Appendix I.

NCI - Cooperative Group - Industry Relationship Guidelines

NCI - Cooperative Group - Industry Relationship Guidelines:
http://ctep.cancer.gov/industry/industry.html

Appendix II.

Special Article: Commentary Concerning Demonstration of Safety and Efficacy of Investigational Anticancer Agents in Clinical Trials

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Abstract

Expeditious clinical development and approval of new drugs that are beneficial to patients are matters of high priority. There has been a great deal of discussion within the oncology community about what should constitute evidence of effectiveness of new anticancer agents for purposes of drug approval. This commentary is intended to illustrate a variety of end points that can lead to approval of new anticancer agents for specific clinical situations. Although the ultimate hope of antineoplastic therapy is prolongation of life, there are other effects of anticancer drugs that constitute clear clinical benefit and represent evidence of effectiveness. The guiding principle is that the beneficial effects obtained from a new drug should sufficiently outweigh the adverse effects such that the potential risk: benefit ratio achieved by an individual patient is favorable. The assessment of a new drug should flexibly evaluate safety and efficacy in the context of the specific clinical condition being treated. Early discussions with the Food and Drug Administration (FDA), http://www.fda.gov/, and the National Cancer Institute (NCI), http://cancer.gov, are recommended to identify prospectively the end points and trial designs needed to demonstrate effectiveness of a new drug. The general principles discussed will likely apply to the drug approval process for other medical disciplines as well.

Introduction

Expeditious clinical development and approval of new anticancer drugs that are beneficial to patients are matters of high priority. There has been a great deal of discussion within the oncology community about what should constitute evidence of effectiveness of new anticancer agents for purposes of drug approval. This commentary represents the efforts of a Working Group composed of Food and Drug Administration (FDA) staff in the :

- Center for Drug Evaluation and Research (CDER), http://www.fda.gov/cder/
- Center for Biologics Evaluation and Research (CBER), http://www.fda.gov/cber/index.html
- National Cancer Institute (NCI), http://cancer.gov
- NCI Division of Cancer Treatment (DCT) Board of Scientific Counselors.

It is intended to illustrate a variety of end points that can lead to approval of new anticancer agents for specific clinical situations.

Assumptions

- The legal requirements for evidence of safety and efficacy are scientifically sound conditions for drug approval. (The words "drug" and "agent" are used interchangeably throughout to mean an investigational, antineoplastic therapeutic, whether a cytotoxic or biologic).
- Neither safety nor efficacy can be defined absolutely in the context of cancer treatment. Anticancer agents cannot always be expected to cure tumors, and they cannot be uniformly safe. The clinical usefulness of a drug must reflect the relationship of risk to benefit for specific clinical conditions (net clinical benefit). In particular, net clinical benefit can be defined for specific clinical situations. For example:
    - Substantial clinical improvement in patients with refractory cancer might result in a favorable risk: benefit ratio (net clinical benefit) despite very substantial toxicity.
    - Modest, reproducible levels of benefit would be considered to confer net clinical benefit if the level of toxicity is modest.
    - For agents with intermediate degrees of benefit and toxicity, judgments regarding net clinical benefit rest ultimately on whether benefits to the treated population outweigh adverse effects.
    - A new drug need not have greater effectiveness than standard treatment to product net clinical benefit. Similarly, analogs do not need to be superior to the parent compound. Indeed, new agents or analogs that avoid significant organ toxicity or that have some other clear advantage may have a favorable risk: benefit ratio even if they have less antitumor activity than a standard agent.
- The primary aim of cancer treatment is prolongation of life, but the demonstration that a new agent causes tumor regression and improves patients' clinical condition also supports approval of a new agent, even in the absence of improved survival.
- Expediting drug approval should not interfere with the full study of a promising new drug.
- Studies vital to a new drug's optimal use should be conducted concurrently with pivotal marketing trials, or as soon as possible thereafter. These would include elucidation of the relationship of dose to response and toxicity, comparison of the new drug to other available treatments, and the drug's utility in various clinical settings.
- Randomized clinical trials in support of a New Drug Application or Product License Application are generally the preferred means of drug evaluation, especially for conditions where standard therapies produce clinical benefit. However, in cases where the beneficial effect is large or dramatic (e.g., long term survivors or improved quality of

life in patients with refractory tumors), results from historically controlled or other nonrandomized trials can provide evidence of substantial clinical benefit.

- The premarketing experience with a drug should be sufficient to characterize its long- and short-term benefits and toxicities. Sufficient numbers of patients should be treated long enough so that uncommon but medically important toxicities, whether acute or chronic, will have a high probability of being detected.
- The effectiveness of a new agent as compared with standard available treatment is a medically important question that can usually be addressed either in the premarketing or postmarketing period in randomized, controlled trials. Comparisons with standard therapies are not necessary for new agent approval if substantial clinical benefit can be established without them (e.g., long-term survivors, high response rates, or improved quality of life in patients with previously refractory tumors).

The precise labeling indication being sought by the sponsor strongly influences whether comparative trials are needed before new drug approval. The new therapy must be compared with standard treatment when the labeling indication sought by the sponsor refers to a patient population where a standard therapy produces cures or significantly prolongs life. In such cases, comparative trials should be done to assess the risks of giving the new therapy, since it may be less effective than standard therapy.

For labeling indications that refer to the treatment of tumors for which there is no life-prolonging or curative therapy, approval of a new agent may not require comparative trials. Comparative trials of significant medical interest can and should be performed in the postmarketing period. For example, it is often possible to establish the usefulness of an agent in refractory or intolerant patients with relatively small studies well before evaluating the role of the agent as front-line treatment. The expeditious path to marketing a new agent may be for sponsors to focus initially on labeling indications for refractory or intolerant patients for whom there is no life-prolonging or curative therapy. This approach was taken in developing etoposide, ifosfamide, carboplatin, and fludarabine. Comparative efficacy trial conducted in the postmarketing period can then address other medically important questions and support other labeling indications.

Approaches to the Assessment of Net Benefit to the Treated Population

The following are acceptable end points for demonstrating effectiveness.

Survival Benefit:

Effectiveness is clearly shown when a new agent imparts a clinically significant survival advantage to the treated population. Such a therapeutic effect is generally best demonstrated in randomized, controlled trials (RCTs), although the effects may even be apparent through comparison with historical controls (e.g., etoposide for the second-line and ifosfamide for the third line treatment of metastatic germ cell tumors).

Time-to-Treatment Failure and Disease-Free Survival:

Improvement in time-to-treatment failure (TTF) or disease-free survival (DFS) in the adjuvant setting is generally best shown in a RCT and can be a reasonable basis for concluding that an agent is effective. For example, in breast cancer a large fraction of recurrences are symptomatic, making improved DFS a valid surrogate for improved quality of life (QOL).

Complete Response Rate:

For a variety of malignancies, an increased rate of durable complete responses (CRs) correlates with increased rates of survival and cure. Thus, reproducible, carefully documented CR rates of reasonable duration are persuasive indicators of effectiveness, particularly if there are no other effective options. On the other hand, when curative, standard therapy exists, it is important to ensure that the CR rate of the new drug translates into a comparable effect on survival.

Response Rate:
It may be argued that virtually any drug should be considered effective if it produces a response rate above some arbitrary level (e.g., 20% for may solid tumors). If one were to accept such thresholds, it seems clear that the level should be a function of the tumor type and stage. For example, the threshold response rate for previously untreated indolent lymphoma might be considerably higher than that for renal cell carcinoma or melanoma. In general, however, it is not meaningful to consider response rate without also considering duration of response, the general level of toxicity, and effects on QOL.

Beneficial Effects on Disease-Related Symptoms and/or Quality of Life (QOL):
QOL may be improved if treatment decreases symptoms of disease, as measured directly or with validated QOL instruments. Parameters of QOL could include improvement in tumor-related signs or symptoms, improved physical or psycho logic function, decreased reliance on medical support, gain in lean body mass, etc. The usefulness of QOL instruments in demonstrating patient improvement is a matter of considerable interest. Improved QOL may be demonstrated in two kinds of studies:

Comparison with control therapy in a Randomized, Controlled Trial(RCT):
For a new drug with substantial toxicity, net clinical benefit can be best shown through a RCT. If the improvement in QOL achieved with the new treatment is at least equivalent to a control treatment also shown to enhance QOL, then the new treatment can be considered effective. On the other hand, the new drug would have to provide superior QOL if compared with a control therapy that did not result in improved QOL. In situations where life-prolonging standard therapy exists, survival would also have to be assessed, as one would not usually sacrifice survival for superior QOL. However, once the comparative impact on survival is clear, individual patients and physicians can consider the relative importance of symptom relief and QOL versus survival.

Comparison of patient's post treatment status with pretreatment status:
In patients who have well-defined symptoms or signs that confidently can be attributed to their tumors, disappearance or amelioration of the symptoms may be a meaningful result. This design has been used recently for interferon alpha in the treatment of hairy cell leukemia; reduction in transfusion requirements and infection rates were used to document treatment effectiveness. Another example is the somatostatin analogs, which reduce symptoms from hormone secretion in islet cell/carcinoid tumors. This trial design is also suitable for evaluating QOL end points in the treatment of rare, symptomatic tumors, where accrual to larger, randomized studies would take a very long time. Because this approach can be confounded by drug toxicity, it is best suited to the evaluation of relatively well-tolerated regimens. This design could not detect a treatment-induced slowing of clinical deterioration because it lacks a comparison group.

Illustrative Examples
It is assumed in these examples that

- The clinical results are derived from prospective studies with samples sizes and methodologies that provide relatively precise estimates of effects, and confidence intervals that assure that clinically significant differences are not missed
- The patient populations are studied are well defined
- The estimated response rates are reproducible
- Standard definitions of response and other end points are used.

These examples show that the expected benefit from a treatment effect depends on the clinical circumstances in which it occurs. In all cases, the guiding principle is that the beneficial effects

obtained should sufficiently outweigh the adverse effects, such that the potential risk: benefit ratio achieved by an individual patient is likely to be favorable. The assessment of a new drug should flexibly evaluate safety and efficacy in the context of the specific clinical condition being treated.

Examples Demonstrating Effectiveness in Clinical Settings where Life-Prolonging or Curative Standard Therapy Exists

Example 1:

A new drug (use singly or in replacing a drug in a standard combination) for front-line treatment of advanced testicular cancer produces identical complete response (CR) rates compared with standard therapy. Data are not yet available comparing survival experience. Toxicity with the new drug is less.

Comments:

To conclude that the new drug is effective for this indication when standard treatment is known to be curative, adequate assurance of comparable 3-year survival and freedom from relapse are required. If the similar response rates correlated with similar survival in one study, conclusions from the confirmatory study could be based on CR rates alone.

Example 2:

Drug B is an analog of parent drug A (such as cisplatin). It is being developed for the treatment of the same tumors for which drug A is used, as well as for others. It differs from A in that some toxicities are reduced. Drug B may or may not be completely cross resistant with drug A.

Comments:

Comparative trials of A and B may or may not be necessary. If A (the parent) is clearly effective in improving survival in a particular tumor type and stage, and B (the analog) is being developed as a substitute for the parent for the same type/stage of disease, comparative trials are necessary. Results should show similar effects on survival or well-established surrogate end points for survival. A somewhat poorer result for the new drug, still yielding evidence of a survival benefit, might support (with accurate labeling) availability for people who are not willing to accept the side effects of the parent drug. For people intolerant of the parent drug or who have a medical condition that precludes the use of the parent drug, evidence of effectiveness of the analog could be shown in noncomparative studies in drug A-intolerant patients. Acceptable end points of effectiveness would include a good rate of durable CRs or improvement in QOL.

Members of the Working Group did not agree entirely on how to apply these concepts to the approval process in diseases for which standard therapy produces profound degrees of cytoreduction, high remission rates, impressive patient benefit, modest improvements in DFS and overall survival, and a small (10% - 20%) cure rate. Examples include previously untreated advanced ovarian cancer and acute myeloblastic leukemia (AML). A majority of the group favored the position that new agents (whether or not they are analogs) labeled for treatment of such conditions should demonstrate equivalent prolongation of survival or equivalence of accepted surrogates for survival (e.g., durable pathologic CR rates in ovarian cancer). The approval criteria for new drugs for these situations should then differ from approval criteria for other conditions, such as estrogen receptor-negative metastatic breast cancer and extensive small-cell lung cancer, where standard therapy perturbs the natural history of the disease to a much less extent and rarely, if ever, produces cures. In these latter diseases, comparative trials would not be required to demonstrate effectiveness for an active new agent, if that agent produced a significant durable CR rate or improved QOL.

A minority of the group favored the position that if the outcome of standard therapy for diseases such as previously untreated advanced ovarian cancer and AML was sufficiently poor, comparative trials should not be required. If a significant number of durable CRs (comparable with the number achieved with standard therapies) could be demonstrated in previously untreated patients in single-arm studies, a new drug could be considered effective. These members would

accept new agents for front-line treatment of these conditions based on reproducible and substantial activity and safety in Phase 2 trials in previously untreated patients.
These are differing views of the issue, "How good are the standard therapies for advanced ovarian cancer and AML?" For cancers where standard therapies are strictly palliative, comparative trials are generally not needed to show effectiveness of a new drug or analog. On the other hand, for cancers where standard therapy is largely curative (e.g., testicular cancer and Hodgkin's disease), there is general agreement that comparative efficacy trials should be done. For clinical conditions between these two extremes (e.g., standard therapy cures a small minority of patients, provides a modes improvement in survival, or improves QOL to a limited extent), the threshold for deciding that a standard therapy is "good enough" to require comparative studies is a matter of judgment and ongoing discussion.
However, there was strong agreement that for clinical conditions where standard therapy clearly cures, equivalence of a new drug should be demonstrated in comparative trials before approval. Because such studies can require many years to complete, approval of a promising new drug can also be sought for the treatment of refractory cancers based on Phase 2 studies.
If B (the analog) can induce CRs of good duration or improve tumor-related symptoms in patients whose tumors have progressed on A (the parent), then B would be considered effective for second-line treatment.
If B is studied for conditions not known to be responsive to A (whether A has or has not been studied), comparisons with A are not needed.

Example 3:
A new drug is compared with the combination of fluorouracil (5-FU) and levamisole for adjuvant treatment of stage III colon cancer. Toxicity is moderate and is similar for the two arms. DFS and point estimates of survival appear equivalent, but at present there are insufficient data to assess equivalence or inequivalence of survival at a statistically significant level. Twenty-five percent of the expected deaths have occurred on the control arm.
Comments:
The new drug cannot be considered effective at present. Equivalence of survival between the new drug and 5-FU/levamisole has not been established at a statistically significant level. Since the combination of 5FU-levamisole has been shown to increase long-term survival compared with no treatment, it should not be replaced with a treatment that might give inferior survival. In general, DFS is an acceptable surrogate for survival provided it reproducibly correlates with improvement in survival for a particular patient population. To date, few studies have shown survival improvement in stage III colon cancer; therefore, it is not yet possible to assess whether DFS can be used as a surrogate for survival. If DFS could be shown to be a reliable surrogate for survival in this disease, then DFS equivalence would be a basis for concluding that the new drug is effective.
If, in this example, the new drug produced a superior reduction in recurrence rates and an improvement in DFS that were statistically significant, the new drug would be considered effective. It could be assumed that survival with the new drug would be at least equivalent to the combination of 5-FU/levamisole. Follow-up of survival would need to be continued and reported after marketing.

Examples Demonstrating Effectiveness in Clinical Settings where No Standard Life-Prolonging or Curative Therapy Exists

Example 4:
An antiestrogen produces a response rate of 30% in previously untreated patients with post-menopausal, estrogen receptor-positive (ER+), metastatic breast cancer (compared with 50% to

60% for tamoxifen). The response rate is 10% to 15% in patients who have previously responded to and then progressed on, all other hormonal therapy. Toxicity is minimal.

Comments:

If the labeling indication centered on hormonally refractory patients, these results would support the demonstration of net clinical benefit if patients benefit could be shown. There may be other instances in which the magnitude, quality, and/or duration of the objective responses are remarkable and the demonstration of symptomatic improvement may not be necessary, but that is not the case for a 10% to 15% response rate. However, for a labeling indication referring to previously untreated ER+ patients, comparison with tamoxifen in an RCT should be required. The 30% response rate in the previously untreated population would not prove effectiveness by itself. Indeed, the 30% response rate suggests an unacceptable risk: benefit ratio for this indication, since the new drug appears significantly less effective than the established agent and has no other advantages.

Example 5:

A new drug for symptomatic chronic lymphocytic leukemia (CLL) refractory to standard therapy produces a response rate of 50%, with 15% CRs. The duration of response is approximately 1 year for the CRs and 6 months for the partial responses (PRs). Considerable Phase 2 experience also shows the new drug to have a reproducible 80% response rate, with 25% CRs in previously untreated patients with CLL and with response durations comparable to those achieved with standard therapies. Toxicity is mild.

Comments:

Such a drug would be considered effective for treatment of CLL refractory to standard treatment, because of the significant rate of durable CRs. In addition, because of the reproducible, high, durable response rates in previously untreated patients, and the mild toxicity (comparable to treatment with standard therapy), this new drug would also be considered effective for front-line treatment of CLL without the need for comparative efficacy trials. Standard treatment for CLL is not curative and is not known to prolong survival, but is believed to have a favorable impact on QOL. Therefore, reasonable assurance that a new drug imparts comparable net patient benefit is a legitimate basis for demonstrating effectiveness. Comparative efficacy trials of medical interest can and should be conducted in the postmarketing period. Had the new drug been associated with significant toxicity and not had any obvious advantage over standard therapy, however, a comparative trial would usually be needed to assess potential differences in QOL and survival.

Example 6:

A new drug has a reproducible, 40% to 50% response rate in adequately sized trials in previously untreated metastatic breast cancer. There are 10 to 15% unequivocal CRs; median duration of the CRs is 10 months. The patient population studied is representative of the general population of patients with metastatic breast cancer. Toxicity is modest; moderate nausea and vomiting and grade 3 myelosupression in 80% of patients. Patients who respond experience improvement in their disease-related symptoms; nonresponders are not adversely affected to any significant degree.

Comments:

This example is potentially controversial because it depends on one's view of the adequacy of available treatment for metastatic breast cancer. In general, however, this new drug appears to confer net clinical benefit based on the durable CR rate and modest toxicity, and because the response rate and CR rate are not notably inferior to alternative standard therapies. Standard combination chemotherapy has not been clearly shown to increase survival, and cures are not expected. Therefore, comparative trials with standard regimens for metastatic breast cancer would not necessarily be required for approval. These comparative efficacy trials, however, are of significant clinical interest and should be conducted along with pivotal marketing trials, or

immediately thereafter. On the other hand, if this new drug were associated with more severe, potentially life-threatening toxicity, or if the CRs were equivocal or less durable, comparison with a standard regimen would usually be needed to assess whether treatment resulted in inferior QOL or survival.

Example 7:
A new drug produces a 20% response rate (all PRs) in metastatic renal cell carcinoma, with a median duration of 4 months; no PR lasts over 6 months. Treatment-related toxicity includes severe, refractory nausea and vomiting, lasting several days after each dose.
Comments:
It is unlikely that the data would support a claim of net patient benefit. If patients with symptomatic liver, lunch, or brain disease experienced decreased symptoms, perhaps net clinical benefit could be shown. It seems more likely, however, that the short response durations and severe nausea and vomiting would preclude a determination that the risk: benefit ratio is favorable.

Example 8:
A new drug has a reproducible 30% response rate in metastatic renal cell carcinoma, including 10% to 15% durable CRs (median duration of the CRs is 12 months). Toxicity is modest.
Comments:
Effectiveness is clear. The durable CRs strongly suggest that patient benefit has resulted from the new drug. In light of the modest toxicity, the CRs provide a basis for concluding that there is a net benefit.

Example 9:
A cytotoxic agent has activity against Hodgkin's disease refractory to mechlorethamine, vincristine, procarbazine, and prednisone/doxorubicin, bleomycin, vinblastine, and dacarbazine (MOPP/ABVD). The associated response rate is approximate 35%. (PRs only). The median duration of response is 5 months, with no response lasting longer than 8 months. Toxicity is moderate: nausea and vomiting last for 2 to 4 hours in 60% to 70% of patients, grade 3 myelosupression in 75%, and urticaria in about 7%.
Comments:
The drug would be considered to have a favorable risk: benefit ratio for use as salvage therapy if the data show net clinical benefit. Comparison with standard therapy in the salvage setting would not necessarily be required, since there is no established standard therapy for this patient group. Data should show that the 30% response rate, with the associated toxicities, translates into overall benefit for the treated population. One way to show this would be to demonstrate improved symptom/sign control in responders compared with pretreatment status, without strong adverse effects in the nonresponders.
There are important advantages to performing RCTs in this setting, especially if the therapy is toxic. Comparing such a new drug with a second therapy believed to have comparable antitumor activity can aid the demonstration of net patient benefit by providing a basis for comparison of response rates, toxicity, and QOL measurements. Also, a comparative trial could detect a beneficial drug-induced slowing of symptom progression; this could not be definitively demonstrated in a single-arm trial.

Examples Demonstrating Effectiveness of New Agents Aimed at Reducing Treatment-Related Toxicities

Example 10:
A hematopoietic growth factor is shown in an RCT to reduce the duration of neutropenia in small-cell lung cancer (SCLC) patients treated with aggressive combination chemotherapy. The duration is reduced from an average of 7 days to an average of 4 to 5 days. This difference results in significantly fewer episodes of febrile neutropenia and hospitalization. Toxicity from the growth factor is mild (fevers, transient bone pain, skin rashes). There is no significant difference in response rates between the two arms.
Comments:
The shortened duration of neutropenia with consequent fewer episodes of febrile neutropenia and hospitalizations provides a basis for concluding that the growth factor is effective in preventing severe infectious complications of chemotherapy. Other patient benefit end points of interest include decreased need for antibiotics and/or shorter hospitalizations, decreased numbers of documented infections, and lessened mucositis.

Example 11:
The hematopoietic growth factor described above also allows delivery of a more dose-intensive treatment, i.e., a greater total dose per unit time or an improved ability to administer a fully intensive established dose. This is shown for extensive-stage SCLC patients in a randomized trial. The chemotherapy regimen is toxic but known to be effective in SCLC. The improved dose intensity is due to quicker recovery of the WBCs after chemotherapy and fewer cycle delays. Again, toxicity of the growth factor is mild. Patients receiving the more dose-intensive regimen have significantly higher CR rates (35% vs. 20%; durations are comparable), which historically have been shown to correlate with survival in this disease. Median TTF is 10 months for the growth factor arm versus 6 months for the chemotherapy-alone arm.
Comments:
The known correlation of CR rate with overall benefit-in this case, survival-supports the conclusion that the growth factor enhances the effectiveness of chemotherapy. Significant improvement in TTF or QOL would also be a justifiable basis for showing effectiveness.

Example 12:
In a randomized trial, a chemoprotector is shown to reduce the incidence of nephrotoxicity and ototoxicity of a known effective regimen in advanced ovarian cancer. Toxicity of the chemoprotector is mild. The two arms are equivalent in overall response rates and in pathologic CR rates.
Comments:
Reduced chemotherapy-induced nephrotoxicity and ototoxicity with no adverse effects on response rates or overall toxicity form the basis for the demonstration of effectiveness. Data on survival should be submitted during Phase 4 (postapproval).

Acknowledgements
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Appendix
This commentary was written as a white paper in February 1988 by the following members of NCI's DCT Board of Scientific Counselors (BSC) Ad Hoc Review Committee for FDA New Drug Approval: Drs. Martin Abeloff, Robert Capizzi, Lawrence Einhorn, B.J. Kennedy, John Laszlo, Brigid Leventhal, Franco Muggia, John Niderhuber (Chairman), Robert Wittes, and Robert Young. This initial white paper was approved by the full DCT BSC in February 1988 and became the basis for ongoing discussions between the NCI and the FDA. In 1990, this document was expanded and put into its final form by the NCI/FDA Working Group. Substantial contributions to this effort were made by Drs. Samuel Broder, Michael Grever, Michael Hawkins, Carl Peck, Jay Siegel, and Kathryn Zoon. A subcommittee of the DCT BSC consisting of Drs. Paul Carbone (Chairman), William Hryniuk, Loretta Itra, and Donald Kufe reviewed and revised the commentary, which was then approved by the full DCT BSC in February 1991. This commentary has also been reviewed by FDA's Office of Health Affairs and Center for Devices and Radiologic Health, http://www.fda.gov/cdrh/index.html.

Appendix III.

Policy Statement: The Conduct of Phase 1 Trials in Children

Introduction
For over four decades, the NCI has supported evaluations of new agents and new treatment approaches for children with cancer. This support has contributed to the identification of curative treatments for over 75% of children with cancer and has allowed children with cancer to have access to a broad range of new anti-cancer agents. However, despite these advances, over 2000 children and adolescents in the U.S. continue to die from cancer each year. New treatment strategies and novel agents are required to identify curative treatments for these patients. An integral component of the NCI research program for children with cancer is the evaluation of new agents in pediatric Phase 1 trials. Phase 1 trials are essential in order for children to benefit from recent advances in molecular biology and agent discovery that have led to the development of new classes of molecularly targeted agents.

Phase 1 trials for children differ in several fundamental ways from those performed in adult populations. Adult Phase 1 trials are usually conducted at single institutions. Because of the relative rarity of cancer in children, pediatric Phase 1 trials can rarely be performed efficiently by a single institution, and for this reason the NCI generally supports multi-institutional pediatric Phase 1 trials. In multi-institutional Phase 1 trials, it is essential that the flow of information between the participating institutions, the Operations/Data Center, the Study Chair, and the NCI be timely and accurate. Pediatric Phase 1 trials also differ from adult Phase 1 trials in the timing of their initiation and in their starting dose. As described in more detail below, it is common practice to begin pediatric Phase 1 trials following completion of the initial adult Phase 1 experience with an agent, and to begin the pediatric Phase 1 trial at approximately 80% of the recommended Phase 2 dose in adults. Additional information about the design and conduct of pediatric Phase 1 trials is available in published position papers and review articles Additional information about the design and conduct of pediatric Phase 1 trials is available in published position papers and review articles (1;2).

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Selection of Institutions for Participation in Phase 1 Trials
Most NCI-sponsored pediatric Phase 1 trials are performed by consortia that include 10-20 institutions [e.g., the Children's Oncology Group (C.O.G.), Phase 1/Pilot Consortium and the Pediatric Brain Tumor Consortium]. The institutions that are members of these consortia are carefully selected based on their experience in developing and participating in early phase trials, their ability to carefully monitor patients treated on Phase 1/pilot studies, their capabilities in reporting clinical data in a timely manner to the Operations/Data Center, their resources for collection of specimens for required correlative and pharmacokinetic studies, and their ability to contribute to the scientific leadership of the Consortium (e.g., pharmacokinetics, pharmacogenetics, and correlative biology). Institutions must be committed to offering patients participation in Phase 1 trials, to timely submission of all required data and blood/tissue specimens, and to compliance with federal regulations for the protection of research subjects.

Timing of Initiation and Starting Dose for Pediatric Phase 1 Trials
Pediatric Phase 1 trials commonly start once the recommended Phase 2 dose has been established in adults. The starting dose for adult Phase 1 trials is often 10% of the dose found to be lethal to 10% of rodents in toxicology studies. This low dose is selected to minimize the risk of severe adverse events among the first humans receiving new agents. Although the initial dose escalations are large in adult Phase 1 studies, it is not uncommon in these studies to evaluate ten or more dose levels before dose-limiting toxicity is reached. Completion of studies with a large number of dose levels requires a substantial number of patients. In contrast, the starting dose for pediatric Phase 1 studies is usually 80% of the adult recommended Phase 2 dose, and the trial escalates in 25-30% increments as successive cohorts of children are accrued to the study. By taking advantage of the adult Phase 1 MTD to determine the pediatric Phase 1 starting dose:

- Pediatric Phase 1 trials commonly require fewer than five dose levels and fewer patients to establish the pediatric MTD.
- The occurrence of unanticipated, severe adverse events at the starting dose levels is minimized, as there is considerable adult experience documenting the agent's adverse event profile.
- All children entered onto a Phase 1 study receive a dose of the agent that is near the adult Phase 2 dose.

This strategy has been successfully employed for over a decade, and in most cases has allowed the efficient determination of a pediatric MTD that is 80% or more of the adult MTD. With this approach, pediatric Phase 1 studies can commence at a relatively early time point in the adult development program of an agent without waiting until completion of the adult development program for the agent. For those agents that achieve target levels in adults without causing dose-limiting toxicity (DLT), the initial pediatric experience can begin at or near the dose in adults that results in the desired biological/clinical effect.

Prioritization of Agents for Phase 1 Evaluation in Children
Approximately 400 new agents are currently under evaluation for cancer indications in adults. Only a small fraction of these can be evaluated in children with cancer as a result of the thankfully small number of children eligible for clinical trials evaluating new agents. Because of this increasing imbalance between the number of new agents potentially available for pediatric evaluation and the number that can actually be evaluated, it is essential to effectively prioritize new anticancer agents for testing in children. The pediatric consortia are charged with developing procedures for making decisions concerning the agents and regimens for which the consortia will develop clinical trials. Data from pediatric preclinical models may provide information that is useful in prioritizing new anticancer agents for testing in children.

Protocol Development and Approval
Pediatric Phase 1 protocols developed by the NCI-sponsored pediatric consortia should be preceded by a written Letter of Intent (LOI) from the Consortium to the CTEP LOI Coordinator declaring interest in conducting a particular study. The LOI should describe the hypothesis to be investigated, the general design of the contemplated trial, plus relevant information on accrual capabilities to document feasibility. Protocols are to be developed and submitted, and studies are to be conducted, in accordance with the DCTD "Investigator's Handbook". The Operations Center for the consortia communicate the results of the NCI's LOI and protocols reviews to the consortia's member institutions and to relevant committees. All protocols utilizing NCI-sponsored investigational agents are to be conducted in accordance with the terms of the "Intellectual Property Option to Collaborators", http://ctep.cancer.gov/industry/ipo.html, and the NCI Standard Protocol Language for Cooperative Research and Development Agreements (CRADAs) and Clinical Trial Agreements (CTAs).

Agent Distribution
For Phase 1 trials utilizing investigational agents distributed by CTEP, agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution at which the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD, through an annual submission of FDA Form 1572, a CV, the Supplemental Investigator Data Form, and the Financial Disclosure Form. If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution. Agent may be requested by completing a Clinical Drug Request (NIH-986) and mailing it to the Pharmaceutical Management Branch, DCTD, NCI, EPN Room 7149, Bethesda, MD 20892 or faxing it to (301) 480-4612.

Study Monitoring:
Pediatric consortia conducting Phase 1 studies are responsible for assuring accurate and timely knowledge of the progress of each study through:

- Establishing procedures for assigning dose level (for Phase 1/dose escalation studies) at the time a new patient is entered, and assuring that the required observation period has elapsed before beginning a higher dose level;
- Registration, tracking, and reporting of attempts to accrue patients who fulfill NIH Guidelines for accrual of women and minorities to clinical trials with appropriate documentation and reporting of accrual as specified by NIH Guidelines;
- Ongoing assessment of case eligibility and evaluability; and ongoing assessment of patient accrual and adherence to defined accrual goals;
- Timely medical review, quality control, and assessment of patient data;
- Rapid reporting of treatment-related morbidity (adverse events) and measures to ensure communication of this information to all parties; and
- Interim evaluation and consideration of measures of outcome, as consistent with patient safety and good clinical trials practice for Phase 1 and pilot studies.

Data and Safety Monitoring Policies
Each pediatric consortia conducting Phase 1 trials must establish a Data and Safety Monitoring Policy in compliance with NIH and NCI guidelines for data monitoring in Phase 1 and pilot

studies. The policy must be approved by the NCI Program Director. Information concerning NIH policy is available at http://grants.nih.gov/grants/guide/notice-files/not98-084.html with additional description at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-00-038.html. Information concerning essential elements of data and safety monitoring plans for clinical trials funded by the NCI is available on the NCI web site, http://www.cancer.gov/, in the "Conducting Clinical Trials" section.

Adverse Event (AE) Reporting

Each pediatric consortia conducting Phase 1 trials is responsible for establishment of a system for assuring timely reporting of all serious and/or unexpected adverse events. For investigational agents sponsored by the NCI, this involves reporting to the Investigational Drug Branch (IDB), CTEP via the AdEERS system, http://ctep.cancer.gov/reporting/adeers.html, according to CTEP guidelines specified in each protocol. Each of the member institutions of the consortia is responsible for implementing the procedures established for assuring timely reporting of all serious and/or unexpected adverse events.

Site Visit Monitoring

The pediatric consortia conducting Phase 1 trials must establish an on-site monitoring program in coordination with the Clinical Trials Monitoring Branch (CTMB, CTEP). For the C.O.G. Phase 1/Pilot Consortium, this involves annual on-site auditing of member institutions by the Clinical Trials Monitoring Service (CTMS). The on-site audit program addresses issues of data verification, protocol compliance, compliance with regulatory requirements for the protection of human subjects, and investigational agent accountability. Any serious problems with data verification or compliance with Federal regulations must be reported to the Clinical Trials Monitoring Branch immediately. The Operations/Data Center will be responsible for coordinating development of and compliance with corrective programs in response to audits.

Pediatric Exclusivity

Section 111 of the Food and Drug Administration Modernization Act of 1997 (the Modernization Act) created section 505A of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. 355a). Section 505A permits certain marketing applications to obtain an additional 6 months of marketing exclusivity (i.e., "pediatric exclusivity") if the applicant, in response to a Written Request from the FDA, files reports of investigations studying the use of the agent in the pediatric population. The pediatric exclusivity provisions were extended until October, 2007 by the *"Best Pharmaceuticals for Children Act (Public Law 107-109)"*. Guidance from the FDA prescribes the general need for pediatric Phase 1 studies as a condition for obtaining "pediatric exclusivity" (see http://www.fda.gov/cder/guidance/3756dft.pdf). The guidance recommends that when planning pediatric protocols, pharmaceutical sponsors should discuss protocol designs with a pediatric cooperative study group, as these groups have experience, expertise, and resources that can help applicants optimize their study designs and accrue patients. The NCI-sponsored consortia for conducting Phase 1 trials, as well as CTEP staff, are available to assist pharmaceutical sponsors in evaluating whether their agents warrant consideration for pediatric exclusivity, and if so, the design of the early phase studies that would be appropriate to conduct in order to obtain exclusivity.

Reference List

(1) Smith M, Bernstein M, Bleyer WA, Borsi JD, Ho P, Lewis IJ, et al. Conduct of Phase I trials in children with cancer. J Clin Oncol 1998; 16(3):966-978.

(2) Bernstein ML, Reaman GH, Hirschfeld S. Developmental therapeutics in childhood cancer. A perspective from the Children's Oncology Group and the US Food and Drug Administration. Hematol Oncol Clin North Am 2001; 15(4):631-655.

Appendix IV.

Key Contact Addresses and Telephone Numbers

For Reporting Adverse Events, use the following address: Investigational Drug Branch Post Office Box 30012 Bethesda, MD 20824 Telephone: (301) 230-2330 Fax: (301) 230-0159	**For Submitting Letters of Intent by USPS, use the following address:** LOI Coordinator, PIO CTEP/DCTD/NCI Executive Plaza North, Room 7000 Bethesda, MD 20892 Telephone: (301) 496-1367 Fax: (301) 496-9384 Email: pio@ctep.nci.nhi.gov
For all other U.S. Mail correspondence, the address is as follows: Cancer Therapy Evaluation Program Executive Plaza North, Room _____ 6130 Executive Boulevard Bethesda, MD 20892	**For Overnight Commercial Carrier, use the following street address:** CTEP/DCTD/NCI Executive Plaza North, Room _____ 6130 Executive Boulevard Rockville, MD 20852

Inquiry Type	Section/ Branch	Room Number	Telephone	FAX
Administrative Inquiries:				
Protocols and Amendments	Protocol and Information Office	7000	301-496-1367	301-496-9384
Investigator's brochures, Clinical Drug Orders, Drug Information, Investigator Registration Information	Drug Management and Authorization Section, Pharmaceutical Management Branch	7149	301-496-5725	301-402-0429
Requests for Nonclinical Use of Investigational Agents, i.e., Group C, Special Exceptions, Treatment Referral Center, Drug Information	Clinical Research Pharmacy Section, Pharmaceutical Management Branch	7149	301-496-5725	301-402-4870
Quality Assurance, Site Visits, Monitoring, and Informed Consent Issues	Chief, Clinical Trials Monitoring Branch	6103	301-496-0510	301-480-2642
Regulatory Questions	Head, Drug Regulatory Affairs Section, Regulatory Affairs Branch	7111	301-496-7912	301-402-1584
Scientific Inquiries:				
Biologic-Related Questions	Head, Biologics Evaluation Section, Investigational Drug Branch	7131	301-496-8798	301-402-0428
Chemotherapy Drug-Related Questions	Head, Developmental Chemotherapy Section, Investigational Drug Branch	7131	301-496-1196	301-402-0428
Disease-Related Questions	Disease Coordinator, Clinical Investigations Branch	7025	301-496-6056	301-402-0557

Appendix V.

Instructions for Completing Statement of Investigator (FDA 1572 Form), Supplemental Investigator Data Form, and Financial Disclosure Form

FDA 1572 Form, the Supplemental Investigator Data Form, and the Financial Disclosure Form for Investigator Registration and instructions for completing these forms are available at:
http://ctep.cancer.gov/forms/index.html

Return the completed forms to:
Pharmaceutical Management Branch, CTEP
Division of Cancer Treatment and Diagnosis, NCI
Executive Plaza North, Room 7149
6130 Executive Boulevard, MSC 7422
Bethesda, Maryland 20892-7422

Appendix VI.

Guidelines for Submitting Letter of Intent and Completing Letter of Intent Forms

Letter of Intent for an Investigational Drug Trial:
http://ctep.cancer.gov/forms/index.html

Instructions:

Group/Institution	All participants should be listed, except for group wide studies
Agent(s) to be supplied by NCI	State the preferred supplier, if any, for agents produced by more than one company.
Other agents to be used in the protocol	Indicate the sponsor for investigational agents, and the source for commercially available agents.
Tumor Type	Include histologies, stages, and grades (as applicable).
Abnormal organ function permitted	List permissible organ functions if outside of normal range (e.g. creatinine, bilirubin, WBC, platelets).
Phase of study	1, 2, 3, or pilot
Treatment plan	Provide a schema or summary including all treatment modalities, and the dose /schedule/route for all agents. For Phase 1 studies, state the dose levels to be studied.
Rationale/Hypothesis	Briefly summarize the rationale for the proposed study, providing references or unpublished data

	supporting it. State the hypothesis or clinical question to be tested. The experimental design must be appropriate to answer the question asked. (Phase 1/2 study designs are not acceptable, separate LOIs should be submitted for each phase).
Laboratory correlates	Give plans (if any) to correlate laboratory parameters with clinical endpoints, dose levels, etc., including statistical considerations. If nonstandard or experimental laboratory assays are to be conducted, specify the rationale(s) for its (their) use; include references and/or unpublished data.
Endpoints/Statistical Considerations	State the endpoint(s) of interest (e.g. response rate, survival, toxicity amelioration, MTD, or quality of life), and specify the level of activity of interest. The sample size should be planned to answer the clinical question with sufficient statistical power (Fleming, *Biometrics,* **38**:143, 1982) For Phase 2 combination trials, specific historical controls should be described and used in sample size planning (Makuch and Simon, *J. Chronic Dis*., **33**:175, 1980)
List competing studies for which this patient population will be eligible	Include planned (LOIs and protocols in review) as well as active protocols.

NOTE: Cooperative group LOIs must be submitted through the group operations office and must be appropriately signed. Proposals that will be submitted for contract credit through the IDB Phase 1 or Phase 2/3 contract mechanism must be signed by the principal investigator for the contract as well as the protocol chair.

LOIs should be submitted electronically to the Protocol and Information Office, PIO@ctep.nci.nih.gov or:

Federal Express Address
LOI Coordinator
Protocol and Information Office
6130 Executive Blvd.
Executive Plaza North, Rm 7000
Rockville, MD 20852

Regular Mailing Address
LOI Coordinator
Protocol and Information Office
CTEP/DCTD/NCI
Executive Plaza North, Room 7000
Bethesda, MD 20892

Appendix VII.

Informed Consent Checklist

Required Elements

The following elements must be present in the informed consent document:

1. Clearly state that the study involves research.
    - State which drug(s), treatment(s), or delivery technique(s) is/are experimental.

- Clarify the study purpose(s) is layman's terms.
- State the patient's expected duration of participation in study (e.g., the patient will be treated until there is evidence that therapy is no longer effective).
- Give a brief description of the procedure(s) to be performed to monitor the patient during study (e.g., X-rays, lab evaluations, etc.). An exhaustive list is not necessary.
- Give a description of the experimental aspect(s) or new delivery techniques(s) of the study.
- State in specific terms the route of administration of each drug (e.g., I.V., oral, continuous infusion, etc.)
- State estimated time of delivery of each drug or time of procedure (e.g., 5 minutes, 30 minutes, 24 hours, etc.).

2. State which risks are attributed to specific drug(s) or procedure(s).

3. Clarify and describe expected benefit(s) to be derived from participation in this study.

4. In general terms, discuss alternative treatment(s) to participation in this study (e.g., conventional chemotherapy, irradiation, hormonal therapy, surgery, etc.).

5. State the extent to which confidentiality of records will be maintained.
   - State that a qualified representative of FDA may inspect patient/study records.
   - State that a qualified representative of the NCI may inspect patient/study records.
   - State that a qualified representative of the collaborating pharmaceutical sponsor may inspect patient/study records.

6. State if compensation for study-related injury will be provided by the institution or other insurer.

   State if emergency treatment of injury will or will not be provided by the institution.

7. Provide space in the form or list the names(s) and number(s) of contact person(s) for research related questions.

   Provide space in the form or list name(s) and number(s) of the contact person(s) (not involved in the research) for patients rights related questions.

8. Clearly state that participation is voluntary.
   - State that refusal to participate will involve no loss of benefits or penalize the patient's care.
   - State that discontinuation of participation in the study will involve no loss of benefits to which the patient is entitled.

Additional Elements

The following elements may be appropriate for some studies:

1. State that unforeseeable or unexpected risk(s) may be involved.
2. State the circumstances under which the patient's participation may be terminated by the investigator without the patient's consent.
3. State that additional costs may be incurred by the patient's participation in the study.
4. State the consequences of the patient's decision to withdraw from the study.

5. State that significant new findings that relate to the patient's treatment will be discussed with the patient.
6. State the approximate number of patients involved in the study.

Suggested Elements

1. State that a copy of the informed consent form shall be given to the patient.
2. The form should be written in layman's terms.
3. Reference to approval by the IRB, NCI, or Cooperative Group may be misleading to the patient.

Please also consult NCI web site, www.cancer.gov, under Conducting Clinical Trials, Guide to Understanding Informed Consent, Safeguards-Simplification of Informed Consent Documents: Templates

Appendix VIII.
Protocol Submission Worksheet

Protocol Submission Worksheet, v 3.1:
http://ctep.cancer.gov/forms/index.html

Appendix IX.

CTEP Glossary

ACTIVATION: The decision by Group/Institution to open an IND study for patient entry (which occurs after CTEP approval).
ACTIVATION AMENDMENT: Any protocol *change* which occurs *after* CTEP approval and *prior* to local activation. Examples: the study is approved by CTEP with recommendations which are incorporated prior to activation; these changes must be listed and submitted to CTEP as an activation amendment.
AE: *Adverse Event* - An "alarming" AE is any serious, fatal, or life-threatening clinical experience in a patient which is thought to be agent related. It must be reported immediately to the drug sponsor. "Other" AEs are reported if that effect has not been described previously.
AMENDMENT: *Any* protocol *change* which occurs *after* activation.
APPROVAL: CTEP approves the protocol in writing when the science and informed consent are acceptable, the IRB documentation is on file (not applicable to Groups), and the agents to be supplied are specified by the Pharmaceutical Management Branch. If recommendations are specified, CTEP expects an "Activation Amendment" to indicate any changes to the approved document.
BLA: *Biologicals License Application* - The formal process by which the FDA makes a biological product generally available to patients and physicians for specific indications. The BLA supercedes the PLA, or Product License Application for biologicals licensing.

BES: Biologics Evaluation Section, http://ctep.cancer.gov/about/idb.html, Investigational Drug Branch (IDB), CTEP, DCTD, NCI.
BRB: Biometric Research Branch, http://linus.nci.nih.gov/~brb/, CTEP, DCTD, NCI.
CANCER CENTER: *An institution* which is designated by NCI as a comprehensive or clinical cancer center and is eligible to conduct IND drug studies.
CCOP: Community Clinical Oncology Program - A cooperative agreement supported program which provides support to community-based oncologists to participate in clinical trials sponsored by the clinical cooperative groups and/or cancer centers. Each CCOP is expected to enter a minimum of 50 patients per year on NCI approved research protocols.
CIB: Clinical Investigations Branch, http://ctep.cancer.gov/about/cib.html, CTEP, DCTD, NCI.
CLINICAL COOPERATIVE GROUPS: Cancer clinical cooperative groups are composed of investigators who join together to develop and implement common protocols. The characteristic of cooperative groups is the central operations and statistical offices which support the administrative requirements of the research and perform central data collection and analysis.
CLINICAL TRIALS MONITORING SERVICE: An organization which receives, reviews, and performs data management tasks on individual patient case report forms for Phase 1 and some Phase 2 NCI investigational drug studies.
CLOSED A: Study is closed to accrual.
CLOSED B: Study is closed to accrual and treatment.
COMPLETE: The study is closed and no patients are being treated or followed for data collection.
COOPERATIVE ONCOLOGY GROUP ASSURANCE: An agreement for the protection of human research subjects filed with the Office for Human Research Protections (OHRP), http://ohrp.osophs.dhhs.gov/index.html, by an institution participating in cooperative group trials.
CRPS: Clinical Research Pharmacy Section, http://ctep.cancer.gov/about/pmb.html, Pharmaceutical Management Branch (PMB), CTEP, DCTD, NCI.
CTEP: Cancer Therapy Evaluation Program, http://ctep.cancer.gov/, DCTD, NCI.
CTMB: Clinical Trials Monitoring Branch, http://ctep.cancer.gov/about/ctmb.html, CTEP, DCTD, NCI.
DCP: Division of Cancer Prevention, http://www3.cancer.gov/prevention/, NCI.
DCS: Developmental Chemotherapy Section, http://ctep.cancer.gov/about/idb.html, Investigational Drug Branch (IDB), CTEP, DCTD, NCI.
DCTD: Division of Cancer Treatment and Diagnosis, NCI, http://www3.cancer.gov/dctd/
DHHS: Department of Health and Human Services, http://www.dhhs.gov/.
DRUG ACCOUNTABILITY RECORD FORM: Form used to maintain records of disposition of NCI investigational drugs. NIH Form-2564, http://ctep.cancer.gov/forms/index.html.
DMAS: Drug Management and Authorization Section, http://ctep.cancer.gov/about/pmb.html, Pharmaceutical Management Branch (PMB), CTEP, DCTD, NCI.
DRAS: Drug Regulatory Affairs Section, http://ctep.cancer.gov/about/rab.html, Regulatory Affairs Branch (RAB), CTEP, DCTD, NCI.
DTP: Developmental Therapeutics Program, http://dtp.nci.nih.gov/, DCTD, NCI.
FDA: Food and Drug Administration, http://www.fda.gov/, DHHS.
FDA 1572: Also referred to as a "Statement of Investigator;" it is a requirement of Section 505(I) of the Food, Drug and Cosmetic Act and 312.1 of Title 21 CFR, that an investigator complete this form as a condition for receiving and conducting clinical studies involving investigational agent(s). It includes the investigator's training and experience and provides for legal certifications, http://ctep.cancer.gov/forms/index.html.
FORM NIH-986: Clinical Drug Request Form, http://ctep.cancer.gov/forms/index.html.
IDB: Investigational Drug Branch, http://ctep.cancer.gov/about/idb.html, CTEP, DCTD, NCI.
IND: Investigational New Drug Application - The legal mechanism under which experimental agent research is performed in the United States. An IND is submitted to the Food and Drug

Administration in order to receive an exception from premarketing approval requirements so that experimental clinical trials may be conducted. Information on the content and format for INDs can be found in the Guidance Document page of the FDA web site, http://www.fda.gov.
INVESTIGATOR: Any physician who assumes full responsibility for the treatment and evaluation of patients on research protocols as well as the integrity of the research data.
INVESTIGATOR'S BROCHURE: This document contains all relevant information about the agent, including animal screening, preclinical toxicology, and detailed pharmaceutical data. Also included, if available, is a summary of current knowledge about pharmacology and mechanism of action and a full description of the clinical toxicities.
LOI: Letter of Intent-An investigator's declaration of interest in conducting a Phase 1, 2, or pilot trial with a specific investigational agent in a particular disease. Approval of the LOI by CTEP commits an investigator to submit a protocol within a specified time frame.
MULTIPLE PROJECT ASSURANCE (MPA): A formal written agreement with the Office of Human Research Protection, http://ohrp.osophs.dhhs.gov/index.html, (on behalf of the Secretary of DHHS) and an institution which conducts or supports a large amount of DHHS-sponsored research involving human subjects. The MPA specifies how the institution will implement the DHHS regulations 45 CFR 46.
NCI: National Cancer Institute, http://www.cancer.gov/, NIH, DHHS.
NDA: New Drug Application - The formal process by which the FDA makes the agent generally available to patients and physicians for specific indications.
NEW DRUG STUDIES GROUP: Highly qualified clinical researchers at an institution specifically approved by IDB to participate in NCI's agent development program.
NIH: National Institutes of Health, http://www.nih.gov/, DHHS.
OHRP: Office of Human Research Protection, http://ohrp.osophs.dhhs.gov/index.html, NIH.
OFFICIALLY FILED: At the time of CTEP approval, the protocol document, the informed consent, or amendment is placed in the "approved" Protocol and Information Office (PIO) file and is distributed to the Drug Management and Authorization Section (DMAS), http://ctep.cancer.gov/about/pmb.html, the Clinical Trials Monitoring Service, the Food and Drug Administration, and/or PDQ, http://www.cancer.gov/search/clinical_trials/.
PDQ: The Physician Data Query, http://www.cancer.gov/search/clinical_trials/, An online database which makes state-of-the-art treatment, directory, and protocol information available to primary care physicians. This database is maintained by the International Cancer Research Data Bank Branch, International Cancer Information Center (ICIC), NCI.
PIO: The Protocol and Information Office, CTEP, DCTD, NCI. PIO manages the protocol and amendment review process, LOIs, and Concepts and maintains the official record of all NCI-sponsored protocols.
PMB: Pharmaceutical Management Branch, http://ctep.cancer.gov/about/pmb.html, CTEP, DCTD, NCI.
PRB: Pharmaceutical Resources Branch, DTP, DCTD, NCI.
PRC: The CTEP Protocol Review Committee reviews and approves all studies involving DCTD investigational agents, Cooperative Group, or CCOP credit.
PRINCIPAL INVESTIGATOR (PI): Name of physician who has organizational and fiscal responsibility for the use of federal funds to conduct a clinical study.
PROTOCOL CHAIR: The scientific coordinator of the study who is responsible for developing and monitoring the clinical study as well as analyzing, reporting, and publishing its results.
QACS: Quality Assurance and Compliance Section, Clinical Trials Monitoring Branch (CTMB), http://ctep.cancer.gov/about/ctmb.html, CTEP, DCTD, NCI.
QUALITY ASSURANCE: The monitoring of a clinical trial to assure the quality of the data that supports scientific conclusions.
RAB: Regulatory Affairs Branch, http://ctep.cancer.gov/about/rab.html, CTEP, DCTD, NCI.

RESEARCH BASE: An institution or cooperative group which assumes a broad range of responsibilities and functions for the support of clinical trials conducted under its name. It supports the investigator in developing, organizing, implementing, and analyzing clinical trials. It assumes responsibility for the quality of the research, both in concept and execution, and has an important role in assuring patient safety.
REVISIONS: Any protocol change which occurs between initial submission and CTEP approval and official filing.
SENIOR CLINICAL INVESTIGATOR: *A physician* in the IDB that is assigned to each IND drug to coordinate its clinical development.
SINGLE PROJECT ASSURANCE (SPA): A formal written agreement with the Office of Human Research Protection (OHRP), http://ohrp.osophs.dhhs.gov/index.html, (on behalf of the Secretary of DHHS) and an institution which does not have Multiple Project Assurance and conducts a DHHS-sponsored research project. The SPA specifies how the institution will implement the DHHS regulations at 45 CFR 46.
SPONSOR: An organization or individual who assumes legal responsibility for supervising or overseeing clinical trials with investigational agents.
TEMPORARILY CLOSED: The decision by a Group, Institution, or NCI to stop patient entry pending study evaluation.

Appendix X.
Clinical Drug Request (NIH - 986)

Clinical Drug Request Form:
http://ctep.cancer.gov/forms/index.html

Appendix XI.
Adverse Event Expedited Reporting System (AdEERS)

Adverse Event Expedited Reporting System (AdEERS) instructions and forms:
http://ctep.cancer.gov/reporting/adeers.html

Appendix XII.
NCI Investigational Drug Accountability Record Form

NCI Investigational Drug Accountability Record Form:
http://ctep.cancer.gov/forms/index.html

Appendix XIII.

National Study Commission on Cytotoxic Exposure: Recommendations for handling Cytotoxic Agents

The mutagenic, teratogenic, carcinogenic, and local irritant properties of many cytotoxic agents are well established and pose a possible hazard to the health of occupationally exposed individuals. These potential hazards necessitate special attention to the procedures utilized in the handling, preparation and administration of these agents, and the proper disposal of residues and wastes. These recommendations are intended to provide information for the protection of personnel participating in the clinical process of chemotherapy. It is the responsibility of institutional and private health care providers to adopt and use appropriate procedures for protection and safety.
See also: http://www.nih.gov/od/ors/ds/pubs/cyto/index.htm

Environmental Protection

- Preparation of cytotoxic agents should be performed in a Class II biological safety cabinet located in an area with minimal traffic and air turbulence. Class II Type A cabinets are the minimal requirement. Class II cabinets which are exhausted to the outside are preferred.
- The biological safety cabinet must be certified by qualified personnel at least annually or any time the cabinet is physically moved.

Operator Protection

- Disposable surgical latex gloves are recommended for all procedures involving cytotoxic agents.
- Gloves should routinely be changed approximately every 30 minutes when working steadily with cytotoxic agents. Gloves should be removed immediately after overt contamination.
- Protective barrier garments should be worn for all procedures involving the preparation and disposal of cytotoxic agents. These garments should have a closed front, long sleeves and closed cuff (either elastic or knit).
- Protective garments must not be worn outside the work area.

Techniques and precautions for use in the class II Biological Safety Cabinet

- Special techniques and precautions must be utilized because of the vertical (downward) laminar airflow.
- Clean surfaces of the cabinet using 70% alcohol and a disposable towel before and after preparation. Discard towel into a hazardous chemical waste container.
- Prepare the work surface of the biological safety cabinet by covering it with a plastic-backed absorbent pad. This pad should be changed when the cabinet is cleaned or after a spill.
- The biological safety cabinet should be operated with the blower on, 24 hours per day - seven days a week. Where the biological safety cabinet is utilized infrequently (e.g. 1 or 2 times weekly) it may be turned off after thoroughly cleaning all interior surfaces. Turn on the blower 15 minutes before beginning work in the cabinet.

- Agents preparations must be performed only with the view screen at the recommended access opening. Professionally accepted practices concerning the aseptic preparation of injectable products should be followed.
- All materials needed to complete the procedure should be placed into the biological safety cabinet before beginning work to avoid interruptions of cabinet airflow. Allow a two to three minute period before beginning work for the unit to purge itself of airborne contaminants.
- The proper procedures for use in the biological safety cabinet differ from those used in the horizontal laminar hood because of the nature of the airflow pattern. Clean air descends through the work zone from the top of the cabinet toward the work surface. As it descends, the air is split, with some leaving through the rear perforation and some leaving through the front perforation.
- The least efficient area of the cabinet in terms of product and personnel protection is within three inches of the sides near the front opening, and work should not be performed in these areas
- Entry into and exit from the cabinet should be in a direct manner perpendicular to the face of the cabinet. Rapid movements of the hands in the cabinet and laterally through the protective air barrier should be avoided.

Compounding Procedures and Techniques

- Hands must be washed thoroughly before gloving and after gloves are removed.
- Care must be taken to avoid puncturing of gloves and possible self-inoculation.
- Syringes and I.V sets with Luer-lock fittings should be used whenever possible to avoid spills due to disconnection.
- To minimize aerosolization, vials containing cytotoxic agents should be vented with a hydrophobic filter to equalize internal pressure, or utilize negative pressure technique.
- Before opening ampules, care should be taken to insure that no liquid remains in the tip of the ampule A sterile disposable sponge should be wrapped around the neck of the ampule to reduce aerosolization Ampules should be broken in a direction away from the body.
- For sealed vials, final agents measurement should be performed prior to removing the needle from the stopper of the vial and after the pressure has been equalized.
- A closed collection vessel should be available in the biological safety cabinet or the original vial may be used to hold discarded excess agents solutions.
- Cytotoxic agents should be properly labeled to identify the need for caution in handling (e.g., "Chemotherapy: Dispose of properly)
- The final prepared dosage form should be protected from leakage or breakage by being sealed in a transparent plastic container labeled "Do Not Open if Contents Appear to be Broken".

Precautions for Administration

- Disposable surgical latex gloves should be worn during administration of cytotoxic agents. Hands must be washed thoroughly before gloving and after gloves are removed.
- Protective barrier garments may be worn. Such garments should have a should have a closed front, long sleeves and closed cuffs (either elastic or knit)
- Syringes and I.V sets with Luer-lock fittings should be used whenever possible.
- Special care must be taken in priming I.V sets. The distal tip or needle cover must be removed before priming. Priming can be performed into a sterile, alcohol-dampened gauze sponge. Other acceptable methods of priming such as closed receptacles (e.g.,

evacuated containers) or back-filling of I.V. sets may be utilized. Do not prime sets or syringes into the sink or any open receptacle

Disposal Procedures

- Place contaminated materials in a leak proof, puncture-proof container appropriately marked as hazardous chemical waste. These containers should be suitable to collect bottles, vials, gloves, disposable gowns and other materials used in the preparation and administration of cytotoxic agents.
- Contaminated needles, syringes, sets and tubing should be disposed of intact. In order to prevent aerosolization, needles and syringes should not be clipped.
- Cytotoxic agents waste should be transported according to the institutional procedures for hazardous material.
- There is insufficient information to recommend any preferred method for disposal of cytotoxic agents waste.
    - One acceptable method for disposal of hazardous waste is by incineration in an Environmental Protection Agency (EPA) permitted hazardous waste incinerator.
    - Another acceptable method of disposal is by burial at an EPA permitted hazardous waste site.
    - A licensed hazardous waste disposal company may be consulted for information concerning available methods of disposal in the local area.

Personal Policy Recommendations

- Personnel involved in any aspect of the handling of cytotoxic agents must receive an orientation to the agents, including their known risks, and special training in safe handling procedures.
- Access to the compounding area must be limited to authorized personnel.
- Personnel working with these agents should be supervised regularly to insure compliance with procedures.
- Acute exposures must be documented, and the employee referred for medical examination.
- Personnel should refrain from applying cosmetics in the work area. Cosmetics may provide a source of prolonged exposure if contaminated.
- Eating, drinking, chewing gum, smoking or storing food in areas where cytotoxic agents are handled should be prohibited. Each of these can be a source of ingestion if they are accidentally contaminated.

Monitoring Procedures

- Policies and procedures to monitor the equipment and operating techniques of personnel handling cytotoxic agents should be implemented and performed on a regular basis with appropriate documentation. Specific methods of monitoring should be developed to meet the complexities of the function.
- It is recommended that personnel involved in the preparation of cytotoxic agents be given periodic health examinations in accordance with institutional policy.

Procedure for Acute Exposure or Spills

Acute Exposure

- Overtly contaminated gloves or outer garments should be removed immediately.

- Hands must be washed after removing gloves. Some cytotoxic agents have been documented to penetrate gloves.
- In case of skin contact with a cytotoxic agents product, the affected area should be washed thoroughly with soap and water. Refer for medical attention as soon as possible.
- For eye exposure, flush affected eye with copious amounts of water, and refer for medical attention immediately.

Spills

- All personnel involved in the clean-up of a spill should wear protective barrier garments (e.g. gloves, gowns, etc.). These garments and other material used in the process should be disposed of properly.
- Double gloving is recommended for cleaning up spills.

Position Statement

The handling of cytotoxic agents by women who are pregnant, attempting to conceive, or breast feeding.

There are substantial data regarding the mutagenic, teratogenic and abortifacient properties of certain cytotoxic agents both in animals and humans who have received therapeutic doses of these agents. Additionally, the scientific literature suggests a possible association of occupational exposure to certain cytotoxic agents during the first trimester of pregnancy with fetal loss, or malformation. These data suggest the need for caution when women who are pregnant, or attempting to conceive, handle cytotoxic agents. Incidentally there is no evidence relating male exposure to cytotoxic agents with adverse fetal outcome.

There are no studies which address the possible risk associated with the occupational exposure to cytotoxic agents and the passage of these agents into breast milk. Nevertheless, it is prudent that women who are breast feeding should exercise caution in handling cytotoxic agents.

If all procedures for safe handling, such as those recommended by the Commission are complied with, the potential for exposure will be minimized.

Personnel should be provided with information to make an individua1 decision. This information should be provided in written form and it is advisable that a statement of understanding be signed.

It is essential to refer to individual state right-to-know laws to insure compliance.

National Study Commission on Cytotoxic Exposure

Chairman

Louis P. Jeffrey, Sc.D. Director of Pharmacy Services, Rhode Island Hospital Providence, Rhode Island 02902

Commissioners

Roger W. Anderson, M.S. Director of Pharmacy, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute	Thomas H. Connor, Ph.D. Assistant Professor of Environmental Sciences, Houston School of Public Health, University of Texas Health Sciences Center at Houston	William E. Evans, Pharm.D. Director, Pharmaceutical Division, St. Jude Children's Research Hospital
Clarence L. Fortner, M.S. Head, Drug Management and Authorization Section,	Joseph E Gallelli, Ph.D. Chief, Pharmacy Department, The Clinical Center, National	Joseph N. Gallina, Pharm.D. Director of Pharmacy Services, University of

IDB,CTEP Division of Cancer Treatment, National Cancer Institute	Institutes of Health	Maryland, Medical System Hospital
Dennis M. Hoffman, Pharm.D. Director of Pharmacy Services, University of New York at Stony Brook	Louis A. Leone, M.D. Director of Medical Oncology, Rhode Island Hospital	Suzanne A. Miller, R.N. Oncology Nurse Consultant
Robert M. O'Bryan, M.D. Division Head, Medical Oncology, Henry Ford Hospital		

For additional information contact:
Louis P. Jeffrey, Sc.D., Chairman
National Study Commission on Cytotoxic Exposure
Massachusetts College of Pharmacy and Allied Health Sciences
179 Longwood Avenue Boston. Massachusetts 02115

Appendix XIV.
Policy for Group C Drug Distribution

http://ctep.cancer.gov/requisition/compassion.html

Policy for Group C Drug Distribution
Group C agents are investigational agents provided by the National Cancer Institute to properly trained physicians for the treatment of individual patients who meet the eligibility criteria. Agents within this category have been approved by the Food and Drug Administration for the treatment of the specific cancer identified in the guideline protocol.
Group C drugs are provided for the treatment of patients as indicated below:

Drug	Approved Group C Use
Azacytidine*	Refractory Acute Myelogenous Leukemia (AML) Single agent use only.

Original Agent request:
Physicians may obtain any agent listed by telephoning the Clinical Research Pharmacy Section, http://ctep.cancer.gov/about/pmb.html, of the Pharmaceutical Management Branch. The telephone number is (301) 496-5725. (Monday - Friday 9 - 4:30pm EST)
Agent Reorders:
Additional agent may be requested by completing a Clinical Drug Request Form and sending it by fax to 301-480-4612. You may only reorder agent for a patient previously registered on this protocol. The patient's first name and initial of last name must be indicated on the Clinical Drug Request Form. A blank request form is enclosed in each agent shipment for use with reorders. Telephone orders will not be accepted.

* The FDA has granted a waiver from local IRB Approval. However, you should check with your IRB to determine whether its local policy requires approval.

National Cancer Institute Procedures for Management of Investigational Agents Acquired for Treatment / Group C Protocol Use in Individual Patients

A) Investigator Registration:

A physician must be registered with the National Cancer Institute as an investigator by having completed a "Statement of Investigator" FDA Form 1572, Supplemental Investigator Data Form (SIDF), Financial Disclosure Form (FDF), and a CV. Forms are available at: http://ctep.cancer.gov/forms/index.html. *If you are NOT currently registered,* a Form 1572, SIDF, and FDF are enclosed, with the understanding you will complete and return these registration forms within 10 working days of their receipt.

B) Informed Consent:

Written informed consent must be obtained from all patients treated with Group C agents and kept on file by the physician. For your convenience, a model informed consent document is included in the enclosed protocol.

C) Institutional Review Board Approval:

NCI has obtained an exemption from FDA eliminating the requirement for IRB approval. However, you should check with your IRB to determine whether its local policy requires approval.

D) Treatment Reports:

You **may** be required to provide patient reports if specified in the enclosed protocol. If required, patient specific forms would be sent to you separately and samples of these forms would be included in the protocol. Complete and return these reports to the

EMMES Corp.: Clinical Trials Support Unit, EMMES Corp., 11325 Seven Locks Rd., Suite 214, Potomac, MD 20854.

E): Group C Protocol Adverse Event Reporting Requirements:

Reporting of adverse events is required for all NCI Special Exception and Group C protocols. The following is a summary of the procedures. For more detailed instructions, computer based training, and the tools used below please see the CTEP home page at http://ctep.cancer.gov/, (click on Reporting Guidelines) or call 301-230-2330.

All reports should be mailed to:

Investigational Drug Branch, P.O. Box 30012, Bethesda, Maryland 20824

Definitions

Adverse Event Expedited Reporting System (AdEERS) – An electronic system for expedited submission of adverse event reports.

Adverse Event - Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.

Attribution – The determination of whether an adverse event is related to a medical treatment or procedure. Attribution categories include Definite, Probable, Possible, Unlikely and Unrelated. When reporting in AdEERS use the patient's first name and last initial for the patient ID in the patient information section.

Procedure

1. Identify the event using the Common Toxicity Criteria version 2.0 (CTC V2.0).

2. Determine the grade or severity of the event by using the CTC criteria. The severity is graded between 1 – 5
3. Determine Attribution of the event (definite, probable, possible, unlikely or unrelated).
4. Determine how the event should be reported by the chart below*:

UNEXPECTED EVENT		**EXPECTED EVENT**	
GRADES 2 – 3 **Attribution of Possible, Probable or Definite**	**GRADES 4 and 5** **Regardless of Attribution**	**GRADES 1 – 3**	**GRADES 4 and 5** **Regardless of Attribution**
Expedited report using AdEERS within 10 working days. (Grade 1 Routine Reporting on treatment summary report).	Report by phone to IDB within 24 hrs. Expedited report using AdEERS to follow within 10 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probably or definite) should be reported with 10 working days.	Routine reporting on the treatment summary report. Expedited reporting not required.	Expedited report, including Grade 5 Aplasia in leukemia patients, within 10 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributes to the agent (possible, probably or definite) should be reported within 10 working days. Grade 4 Myelosuppression not to be reported, but should be submitted as part of the treatment summary report.

- *For **Hospitalization** only - Any medical event equivalent to CTC Grade 3, 4, 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of expected or unexpected and attribution.
- Telephone reports to the Investigational Drug Branch at 301-230-2330 available 24 hours daily (recorder between 5 pm and 9 am EST).
- Expedited reports are to be submitted using AdEERS or the paper templates available at http://ctep.cancer.gov/. The NCI Guidelines for expedited adverse event reporting are also available at this site. If paper templates are used, they should be mailed to Investigational Drug Branch (IDB), PO Box 30012, Bethesda, MD 20824 or by fax to 301-230-0159.
- A list of agent specific expected adverse events will be included in the agent shipment.

F) Investigational Agent Accountability:

NCI Investigational Drug Accountability Records must be maintained for Group C agents and kept on file by the physician

G) Quality Assurance:

Records to confirm that the patient has been treated according to the Group C protocol must be maintained by the physician. NCI or FDA may have access to these records upon request.

H) Failure to comply with any of the above procedures may result in suspension of investigator status and prevent further agent shipments.

I) Agent Reorders:

Additional agent may be requested by completing a Clinical Drug Request Form. You may only order more agent for the patient specifically named on this protocol. The patient's first name and initial of last name should be indicated on the Clinical Drug. Request. A blank request form is enclosed in each agent shipment. Telephone orders will not be accepted.

Appendix XV.
National Cancer Institute Procedure of Investigational Drugs Acquired for Compassionate (Special Exception) Treatment of Individual Patients

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Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require the following steps to be completed as indicated:

1) Investigator Registration:

A physician must be registered with the National Cancer Institute as an investigator by having completed a "Statement of Investigator" FDA Form 1572, Supplemental Investigator Data Form (SIDF), Financial Disclosure Form (FDF), and a CV. Forms are available at: http://ctep.cancer.gov/forms/index.html. *If you are NOT currently registered,* a Form 1572, SIDF, and FDF are enclosed, with the understanding you will complete and return these registration forms within 10 working days of their receipt.

2) Protocol:

A brief protocol must be submitted for each patient which describes the treatment plan, toxicity, efficacy, and monitoring procedures. For your convenience we have devised a standard protocol form which is included and must be completed. The original must be returned to the Pharmaceutical Management Branch, 6130 Executive Boulevard, Room 7149, Bethesda, MD, 20892, within 10 working days. Please retain a copy for your records.

3) Institutional Review Board Approval:

You must obtain Institutional Review Board Approval *prior* to treatment of the patient and retain documentation of this approval in the patients medical record.

4) Informed Consent:

You must obtain a written informed consent which must be signed by the patient or their guardian *prior* to treatment. The informed consent must be retained in the patient's medical record. The informed consent should include a reasonable statement about the potential side effects of the agent. The informed consent must address each of the eight elements required under FDA regulations, as detailed on the accompanying sheet.

5) Final Patient Report:

Upon completion of therapy you must provide NCI a report of the treatment experience. which describes toxicity and efficacy. We have enclosed the form, The Report of the Independent Investigator, to be used. Please return this form to the Clinical Research Pharmacy Section,

Pharmaceutical Management Branch, 6130 Executive Boulevard, Room 7149, Bethesda, MD, 20892.

6) Adverse Events:
Reporting of adverse events is required for all NCI Special Exception and Group C protocols. The following is a summary of the procedures. For more detailed instructions, computer based training, and the tools used below please see the CTEP home page at http://ctep.cancer.gov/ (click on Reporting Guidelines) or call 301-230-2330.
All reports should be mailed to:
Investigational Drug Branch, P.O. Box 30012, Bethesda, Maryland 20824

Definitions
Adverse Event Expedited Reporting System (AdEERS) – An electronic system for expedited submission of adverse event reports.
Adverse Event - Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.
Attribution – The determination of whether an adverse event is related to a medical treatment or procedure. Attribution categories include Definite, Probable, Possible, Unlikely and Unrelated.
When reporting in AdEERS use the patient's first name and last initial for the patient ID in the patient information section.
Procedure
1. Identify the event using the Common Toxicity Criteria version 2.0 (CTC V2.0).
2. Determine the grade or severity of the event by using the CTC criteria. The severity is graded between 1 – 5
3. Determine Attribution of the event (definite, probable, possible, unlikely or unrelated).
4. Determine how the event should be reported by the chart below*:

UNEXPECTED EVENT		EXPECTED EVENT	
GRADES 2 – 3 **Attribution of Possible, Probable or Definite**	**GRADES 4 and 5** **Regardless of Attribution**	**GRADES 1 – 3**	**GRADES 4 and 5** **Regardless of Attribution**
Expedited report using AdEERS within 10 working days. (Grade 1 Routine Reporting on treatment summary report).	Report by phone to IDB within 24 hrs. Expedited report using AdEERS to follow within 10 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probably or definite) should be reported with 10 working days.	Routine reporting on the treatment summary report. Expedited reporting not required.	Expedited report, including Grade 5 Aplasia in leukemia patients, within 10 working days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributes to the agent (possible, probably or definite) should be reported within 10 working days. Grade 4 Myelosuppression not to be reported, but should be submitted as part of the treatment summary report.

- *For **Hospitalization** only - Any medical event equivalent to CTC Grade 3, 4, 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of expected or unexpected and attribution.
- Telephone reports to the Investigational Drug Branch at 301-230-2330 available 24 hours daily (recorder between 5 pm and 9 am EST).
- Expedited reports are to be submitted using AdEERS or the paper templates available at http://ctep.cancer.gov/. The NCI Guidelines for expedited adverse event reporting are also available at this site. If paper templates are used, they should be mailed to Investigational Drug Branch (IDB), PO Box 30012, Bethesda, MD 20824 or by fax to 301-230-0159.
- A list of agent specific expected adverse events will be included in the agent shipment.

7) Investigational Drug Accountability:
Investigational drug accountability records (form enclosed) must be maintained and retained in your records. These records may be inspected upon request by an authorized representative of the FDA, NCI or agent manufacturer.

8) Failure to comply with any of the above procedures may result in suspension of investigator status and prevent further agent shipments.

9) Agent Reorders:
Additional agent may be requested by completing a Clinical Drug Request Form. You may only order more agent for the patient specifically named on this protocol. The patient's first name and

initial of last name should be indicated on the Clinical Drug. Request. A blank request form is enclosed in each agent shipment. Telephone orders will not be accepted.

Appendix XVI.
Guidelines for Treatment Regimens
EXPRESSION AND NOMENCLATURE

Introduction, Policy, General Guidelines, Parenteral Administration, Oral Administration, Concomitant (Ancillary) Medications, Treatment Modifications

INTRODUCTION

All protocols sponsored by the Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI), are reviewed for safety and scientific integrity. Cancer Therapy Evaluation Program (CTEP) staff have developed standardized guidelines to express chemotherapy regimens in a uniform, clear and consistent manner. The intention of the guidelines is to minimize undue risks to patients on DCTD sponsored investigational clinical trials. **DCTD-sponsored protocols will not be approved unless they comply with the *Guidelines for Treatment Regimen Expression and Nomenclature***. CTEP will screen all protocol related documents to assure compliance with the treatment regimen guidelines. These guidelines should be used in all facets of protocol development including Letters of Intent (LOIs), Concept Reviews, protocols, protocol amendments, protocol related publications and any other protocol related correspondence.

The development and utilization of a clear and consistent method for expressing chemotherapy dosage schedules and treatment regimens is an important public health issue. Recent events have heightened awareness and concern among medical professionals and the general public to the potential for adverse and fatal outcomes as a consequence of medication errors with oncology agents. The American Society of Health-System Pharmacists (ASHP), the American Medical Association (AMA), and the American Nursing Association (ANA) have recommended systematic standardized approaches to reducing medication errors which include educating health care providers and patients regarding appropriate agent therapy, improved collaboration between health care providers, establishing dosage limits, and standardizing a prescribing vocabulary. The *Guidelines for Treatment Regimen Expression and Nomenclature* are intended to supplement and reinforce the AMA, ANA, and ASHP recommendations with specific examples illustrating how the guidelines can be applied during protocol development.

Comments and recommendations regarding the treatment regimen guidelines were solicited from clinical pharmacists from comprehensive cancer centers, home infusion services, industry and the Cooperative Group pharmacy chair. Guidelines for expressing dose regimens in treatment plans, agent orders, physician notes and product labeling have also been developed. Investigators should refer to *Standardized Guidelines for Treatment Regimens Expression and Nomenclature*, ASHP 1997, for additional information on this topic.

POLICY

Instructions for dose regimens should be complete, clear, and simple to follow. Treatment regimens should be expressed accurately, completely and consistently throughout a protocol document.

GENERAL GUIDELINES

- **Do not abbreviate** agent names or treatment schedules. Abbreviations can be misinterpreted.
- Use complete approved **generic agent names.** Brand names and abbreviations are not acceptable (*e.g.,* specify 'carboplatin' instead of *CBDCA*, 'cisplatin' instead of *CDDP*).

- **Treatment instructions should be explicit**. No detail (no matter how minor) should be omitted; however, avoid unnecessary redundancy.
- **Delete extraneous information** that may confuse readers (*e.g.,* protocols that use only injectable agents products should not include information for a tablet formulation).
- **Use consistent notation** in expressing quantifiable units, (ex. either; 1mcg or 1ug or 1mg; qid or Q6h; kg or m^2)
- **The word, "Units"** should be spelled out to avoid confusion; a letter "U" can be easily mistaken for a zero and may result in a 10-fold overdose.
- **Decimal Points** -
    - Never trail a whole number with a decimal point followed by a zero (*i.e.,* "5 mg" not "5.0 mg"). The decimal point may not be seen, resulting in a 10-fold overdose.
    - In expressing units that are less than the whole number one, the dosage should be written with a decimal point preceded by a zero (*i.e.,* "0**.**125 mg" not "**.**125 mg"). Without the 'zero' prefix, the decimal point may be missed resulting in a dosing error.
- **Body weight** - Agent dosages may be expressed as a function of body surface area, body weight, or may be calculated to produce a pharmacokinetically-targeted endpoint (*e.g.,* serum or plasma concentration or area under the curve [AUC]). Treatment plans should specify whether absolute (*i.e.,* actual), ideal, or lean body weight is used in calculating agent dosage as a function of body weight. In addition, an equation describing how that value is calculated should appear in the treatment plan if agent dosage is a function of a calculated ideal or lean body weight. If agent dosage is a function of a calculated pharmacokinetic endpoint, the equation(s) describing how that value is calculated should also appear in the treatment plan.
- **Contiguous treatment days** - Treatment plans should specify the total number of days a agent is administered and the cycle day that treatment commences. Include parenthetically the cycle days on which treatment occurs.
- **Non-contiguous days** - Treatment plans should specify the cycle days on which each dose should be given.
- **Cycle (or Course) duration** - Treatment cycle duration (or length) should be specified. When a treatment regimen is 21 days in duration, the regimen will be repeated on the twenty-second, forty-third, sixty-fourth…, etc. days following treatment initiation.
- **Duration of administration:**
    - Administration duration should be clearly indicated. If a agent is to be administered on more than one day per cycle, each cycle day should be explicitly identified.
    - "Day One" typically describes the day on which treatment commences when treatment day enumeration is arbitrary. Avoid using *'day 0 (zero)'* when describing treatment schedules unless it is necessary (*e.g.,* when describing the day on which hematopoietic progenitor cells are administered after a cytotoxic conditioning regimen in transplantation protocols).
- **Clarify total dose planned per treatment course** - In all treatment plans (protocols) and agent orders, identify and append parenthetically the total dose (as a function of body weight or surface area) that patients are to receive during a treatment course (or cycle).
- **Administration Dates and Times** - When appropriate include specific starting days and times. Directions indicating events for the twelve o'clock hours should be explicitly expressed (spell out) "12:00 noon" and "12:00 midnight." Expressing time by 24-hour clock notation ('military time') likewise precludes errors due to ambiguous 'a.m.' and 'p.m.' time notations.

- **Treatment information should contain the following elements:**

	Agent Name	**Dosage**	**Administration vehicle name and volume**	**Administration route**	**Administration Instructions**
Example 1	ABC	200 mg/m^2	0.9% sodium chloride injection 500 ml	Intravenously	Over 1 hour
Example 2	XYZ	50 mg/m^2	NA	Orally	With food

	Administration Schedule	**Number of doses to administer, treatment duration, or date when treatment should be discontinued**	**Starting dates (and times when appropriate)**	**Total amount of agent administered per course (expressed parenthetically)**
Example 1	Every 12 hours	For 6 doses	Start on Day 1	(total dose/cycle = 1,200 mg/m^2)
Example 2	Every morning	For 14 days	Start on Day 1	(total dose/cycle = 700 mg/m^2)

PARENTERAL ADMINISTRATION

- Agent products should be prepared within documented stability and sterility guidelines in accordance with practitioners' local clinical and institutional policies and procedures. Agent containers should be changed at least daily unless extended stability and sterility data are available.
- In protocol descriptions and orders for treatment, agent dosage should be expressed as the total amount of agent that will be administered from a single agent container, *i.e.,* the total amount of agent per syringe, bag, or other container that will be dispensed. An exception to this rule applies to agent products with extended stability, where an agent is administered from a single container for greater than 24 hours. In such cases, treatment plans and prescribers' orders should specify the amount of agent that is administered during each 24-hour interval. Product container labels should always identify the amount of agent within the container.
- For agent admixtures that can be prepared in more than one way, practitioners should institute *a priori,* standard and consistent methods governing how each agent will be prepared and administered.
- Include specific fluid volumes and types when possible.

EXAMPLES

Bolus infusion (administration duration ≤ 24 hours):

- Express the amount of agent per container.
- Include the rate of administration, the infusion duration, and days on which the agent is to be administered.

example

"XYZ" 15 mg/m2 diluted in 50 mL 0.9% sodium chloride injection, infuse intravenously over 15 minutes for one dose on day 1 (total dose/cycle = 15 mg/m2)

Agent products stable for ≥ 24 hours - (Containers are prepared daily):

- Express the dose per container.

- Include the total dose (as a function of BSA, weight, etc., when appropriate) in parentheses.
- State that the agent must be prepared daily.

example
"XYZ" 8 mg/m2 per day diluted in 50 mL 0.9% sodium chloride injection, administer by continuous intravenous infusion over 24 hours, daily for three days starting on day 1 (days 1, 2, and 3; total dose/cycle = 24 mg/m2 over 72 hours). A new IV bag should be prepared daily for 3 days.

Agent products stable for ≥ 24 hours - (Containers are prepared for multiple days):

- Express the dose as the amount of agent administered per day and indicate the number of days for which it is administered.
- Include the total dose (as a function of BSA, weight, etc., when appropriate) in parentheses.
- State that this is a multi-day preparation and for how long the preparation should be infused.

example
"XYZ" 8 mg/m2 per day diluted in 50 mL 0.9% sodium chloride injection, by continuous intravenous infusion for three days starting on day 1 (total dose = 24 mg/m2 over 72 hours). This is a multi-day infusion to be infused over 72 hours.

Continuous infusions that require multiple agent product containers:

- Express the dose per container.
- Include the total dose (as a function of BSA, weight…, etc., when appropriate) in parentheses.
- Include the total number of containers used per day.

example
"XYZ" 1 mg/m2 diluted in 50 mL 0.9% sodium chloride injection, administer by continuous intravenous infusion over three hours, every three hours for three days, starting on day 1 (8 bags/day, total dose = 24 mg/m2 over 3 days)

ORAL ADMINISTRATION

- Describe agent dosages and schedules as the amount of agent that will be given (or taken) each time the agent is administered, not as a total daily dose that will be given (or taken) in divided doses, (e.g. 20 mg orally every 6 hours for 5 days vs. 80 mg per day, given in four divided doses for 5days
- Include guidelines regarding 'rounding-off' doses to the nearest capsule or tablet size. Although breaking a tablet into halves at best approximates an accurately measured dose, treatment plan rounding-off rules should indicate whether tablet formulations should be broken to deliver a calculated dosage.
- Whenever possible, include instructions about whether agents should be administered (or taken) with food and any dietary restrictions.

CONCOMITANT (ANCILLARY) MEDICATIONS

- Supportive care and essential ancillary medications required by a treatment regimen should be clearly identified.
- Complete instructions including appropriate indication, dosage, administration route, schedule, restrictions to use, and any other relevant data should be explicitly stated.

TREATMENT MODIFICATIONS

- Treatment plans should explicitly identify when treatment (typically dosage) modifications are appropriate.

- Treatment modifications and the factors predicating treatment modification should be explicit and clear.
- All treatment modifications should be expressed as a specific dose rather than as a percent of the starting dose.

Appendix XVII.
Status of the NCI Preclinical Antitumor Agent Discovery Screen, Principles and Practice of Oncology Updates,

Michael R. Boyd, M.D., Ph.D.

Principles & Practice of Oncology, Vol. 3, No. 10, Oct. 1989

INTRODUCTION

Great strides have been made in the effective treatment of some forms of cancer by means of chemotherapy used alone or in combination with other modalities. Unfortunately, however, the number of available clinically active antitumor agents remains quite small and the spectrum of clinical antitumor activity is generally rather limited. Physicians are thereby constrained in their attempts to exploit further the use of antitumor drugs in the treatment of more resistant, and frequently more common, forms of cancer. Yet, the ultimate potential of chemotherapy in cancer treatment still undoubtedly remains unrealized, and the abundant precedents for effective treatment or cure of certain leukemias, lymphomas, and some other relatively rare forms of cancer continue to provide the impetus for the search for new active agents. To address this challenge, the U.S. National Cancer Institute (NCI) is exploring a new investigational drug screening and drug discovery strategy that departs substantially from the past. The purpose of the present paper is to review briefly the background, rationale, technical challenges, and current progress of this NCI project (for previous reviews and other background information, see refs. 1–11).

STRATEGIES FOR SCREENING AND NEW DRUG DISCOVERY

ROLE OF THE NCI IN ANTICANCER DRUG DISCOVERY

Tens of thousands of new potential anticancer substances are synthesized or isolated every year. The goal of the NCI screening program is to facilitate the selection and prioritization of the very few most promising and novel new agents that can be developed within the available federal and/or private resources. To meet this goal, the NCI has, for many years, provided a resource for the preclinical screening of compounds submitted by grantees, contractors, pharmaceutical and chemical companies, and other individuals and institutions, public and private, worldwide. In so doing, the NCI program has played a substantial role in the discovery and development of many of the available commercial and investigational anticancer agents.[12] The evolving new NCI drug discovery strategies described herein could likewise have substantial impact upon the kinds of new agents discovered, selected, developed, and ultimately becoming available for future clinical evaluation. As described herein, the prospects indeed appear promising. However, preclinical anticancer drug screening is an uncertain science, and all new screening models must be viewed as investigational until ultimately validated, or proved invalid, by appropriate clinical data.

IN VIVO ANIMAL TUMOR SCREENING MODELS

A majority of currently available anticancer agents were initially selected and developed based on activity in a variety of *in vivo* animal cancer screens used by the NCI. Predominant among these screens have been the mouse leukemias, L1210 and P388. From 1975 to 1985, the P388 leukemia model was employed as the initial ("Stage I") screen by the NCI program. Agents initially found active or curative against the P388 typically were tested further ("Stage II") against a panel of four to eight other tumor models, including animal tumors and a few human tumor xenografts. Agents showing potent, broad-spectrum antitumor activity in Stage II tumor panel evaluations were generally given the highest priority for preclinical development and clinical testing. The initial strategy for clinical evaluation (Phase I/II) of such agents generally has been to screen the drug initially in a population of cancer patients representing a diversity of cancer types, then followed by more detailed or comparative evaluations (Phase II/III) in specific tumor types. Thus, a majority of current investigational anticancer agents were initially selected by a disease-specific primary screen *(e.g.,* a single murine leukemia model), further prioritized by a disease-oriented Stage II preclinical screen *(i.e.,* multiple tumor models), followed by disease-oriented clinical screening (multiple tumor types), and, finally, by disease-specific clinical evaluation (Figure 1).

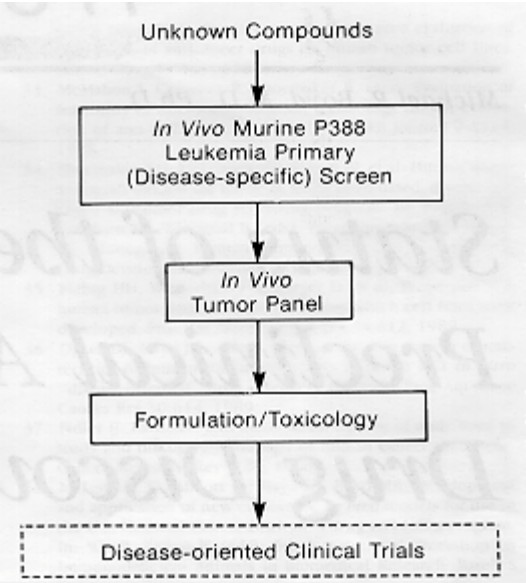

Fig. 1. Compound-oriented preclinical drug development strategy used by NCI 1975-1985

Perhaps a crucial flaw in the above strategy rests on the use of a single disease-specific model in the primary screen. Does it too rigidly filter out those agents with potential specificity against tumors other than mouse leukemia or closely related human diseases? Is it coincidental or irrelevant that a majority of "clinically active" drugs selected and tested in this strategy are active only in leukemia/lymphoma and not in a majority of other types of cancer? Is a disease-specific primary screen the logical precedent for a disease-oriented Stage II preclinical screen and a disease-oriented clinical screen? In an attempt to examine some of these questions, the NCI has been evaluating the feasibility of an alternative *disease-oriented preclinical* anticancer drug discovery strategy aimed at the elucidation of new agents for *disease-specific clinical* trials in relevant cancer patient populations.[1-11]

IN VITRO HUMAN TUMOR DRUG SCREENING MODELS

The disease-oriented strategy necessitates use of a disease-oriented primary screen. Such a screen should employ multiple disease-specific *(e.g.,* tumor-type specific) models and should allow detection of either broad-spectrum or disease-specific activity (Figure 2). Disease-specific Stage II preclinical screens then should provide the logical follow-up, as

well as the optimal interface for early disease-specific (tumor-type specific) clinical trials. Finally, any decision to undertake ultimately a broader clinical screening of an agent against multiple disease types could be soundly based on the accrued preclinical and clinical tumor response data.

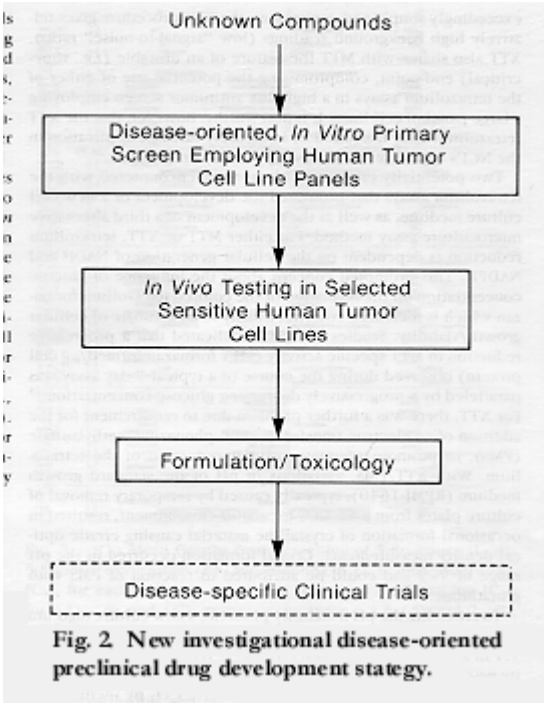

Fig. 2 New investigational disease-oriented preclinical drug development stategy.

The use of multiple *in vivo* animal models for a disease-oriented primary screen is not practical, given the scope of requirements for adequate screening capacity and specific tumor type representation. *In vitro* models, therefore, appear to be the only reasonable alternative. In 1985, the NCI initiated the current project to explore the feasibility of using established human tumor cell lines for a disease-oriented primary drug screen.[1-3]
There had been a previous attempt to use fresh human tumor cells for primary drug screening with the human tumor colony forming assay (HTCFA); however, such an approach proved unfeasible and was ultimately abandoned.[13] The HTCFA and other related assays, nonetheless, remain potentially attractive for use in disease-specific Stage II screening, and also perhaps ultimately for individualization of therapy with a new generation of drugs with greater potential for tumor type specificity.

The availability of a wide variety of human tumor cell lines representing many different forms of human cancer seemed to offer an attractive basis for development of a disease-oriented *in vitro* primary screen. Moreover, since many established human tumor cell lines could be propagated *in vivo* in athymic, nude mice, there appeared the basis for an ideal disease-specific Stage II preclinical screening strategy. Thus, the basic concept for the new experimental primary drug screen required an *in vitro* primary screen comprised of a diverse panel of human tumor cell lines arrayed in disease-specific subpanels. Cytotoxic and/or growth inhibitory drug effects on such a panel should be classifiable as to nonspecific *(e.g.,* general cell poison) vs. specific *(e.g.,* with respect to either individual cell lines or groups of cell lines). In Stage II testing, compounds of interest *(e.g.,* showing line- or panel-specific differential cytotoxicity) could be further evaluated *in vivo* in selected sensitive cell lines from the primary screen.

DEVELOPMENT OF THE HUMAN TUMOR CELL LINE *IN VITRO* PRIMARY DRUG SCREENING MODEL

SCOPE OF THE EFFORT

Our efforts to evaluate the feasibility of the *in vitro* primary screen have focused heavily on the development of the cell line panel,[14-20] the investigation of various alternative

assays of *in vitro* drug sensitivity,[20-25] and the implementation of appropriate data management technology. In describing our progress on the *in vitro* primary screening model, it is important to emphasize the scope of the required laboratory tasks and the technical challenges therein. Assuming that the ultimate cell panel consisted of 120 cell lines, against which duplicate tests were performed on 10,000 compounds/year, each of the latter tested at five different concentrations, then the number of individual culture-well assays performed daily is 50,000 or more.

THE CELL LINE PANEL

A total of 60 human tumor cell lines, derived from seven cancer types (lung, colon, melanoma, renal, ovarian, brain, and leukemia) that adequately meet minimal quality assurance criteria (testing for mycoplasma, MAP, human isoenzyme, karyology, *in vivo* tumorigenicity), which are adaptable to a single growth medium and which have reproducible profiles for growth and drug sensitivity, were selected for use in pilot-scale screening operations in 1989.[16,20,26] Mass stocks of each of the lines were prepared and cryopreserved; these stocks provide the reservoir for replacement of the corresponding lines used for drug screening after no more than 20 passages in the screening laboratory.[26]

While many of the current lines are well known and widely used, the clinical history and original tumor pathology of many of the lines are incomplete or unavailable. All cell lines in the interim panel have nevertheless been subjected to detailed, specialized characterizations *(e.g.,* histopathology, ultrastructure, immunocytochemistry) to verify tissue and tumor type. Moreover, parallel projects are underway for the acquisition of better and more diverse candidate cell lines and for the development of new lines directly from surgical specimens or from nude mouse xenografts for which the corresponding clinical backgrounds are more complete. Special focus is also being placed on major cancer types *(e.g.,* breast and prostate) that currently are not represented at all in the panel due to unavailability of suitable lines. During 1990 we anticipate that the panel will be fully expanded to include ten to 12 different major cancer types with a minimum of ten lines in each subpanel.

***IN VITRO* MICROCULTURE ASSAYS FOR CELL GROWTH/VIABILITY**

We have investigated extensively three alternative assays for cellular growth and viability in the primary screen.[20-22, 25-27] Two are metabolic assays; the cellular reduction of a colorless tetrazolium salt (MTT or XTT) yields a colored formazan derivative in proportion to viable cell number. The formazans can be measured conveniently in an automated colorimeter. Details of the MTT and XTT microculture assays have been published elsewhere.[20,22]

The development of the XTT tetrazolium assay was stimulated by the desire to simplify further the MTT procedure by eliminating an aspiration/solubilization step; the reduction of MTT yields an insoluble formazan which must be dissolved in DMSO prior to colorimetry. The XTT reagent is metabolized by viable cells to a water-soluble formazan, allowing the direct reading of optical density in the culture wells without further processing. While exceedingly simple and convenient, the XTT procedure gives

relatively high background readings (low "signal-to-noise" ratio). XTT also shares with MTT the feature of an unstable *(i.e.,* time-critical) end-point, compromising the potential use of either of the tetrazolium assays in a high-flux antitumor screen employing a large panel of cell lines. It is noteworthy, however, that the XTT tetrazolium assay has found an immensely valuable application in the NCI's high-flux AIDS-antiviral primary drug screen.[28,29]

Two potentially critical problems were encountered with the tetrazolium assays that prompted the development of a new cell culture medium, as well as the development of a third alternative microculture assay method. For either MTT or XTT, tetrazolium reduction is dependent on the cellular generation of NADH and NADPH. This prompted concern about the influence of glucose concentration on the formation of the colored tetrazolium formazan which is measured colorimetrically as an estimate of cellular growth/viability. Studies with MTT indicated that a progressive reduction in MTT specific activity (MTT formazan formed/μg cell protein) observed during the course of a typical 7-day assay was paralleled by a progressively decreasing glucose concentration.[23] For XTT, there was a further problem due to requirement for the addition of an electron transfer reagent, phenazine methylsulfate (PMS), to promote adequate cellular reduction of the tetrazolium. With XTT/PMS, variations in pH of the standard growth medium (RPMI-1640), typically caused by temporary removal of culture plates from a 5% CO_2 incubator environment, resulted in occasional formation of crystalline material causing erratic optical density measurements. Crystal formation occurred in the pH range of 7–9 and could be attributed to reaction of PMS with glutathione.[23]

To eliminate the pH instability problem, a new culture medium was developed.[24] The medium has a stable physiological pH of 7.4 in atmospheric CO_2 (0.04%) and derives its buffering capacity primarily from β-glycerophosphate. The new medium was optimized to facilitate growth in atmospheric CO_2 by inclusion of (1) biotin; (2) L-asparagine; and (3) pyruvate and oxaloacetate for metabolic stimulation of intracellular CO_2 production. With either the MTT assay or a non-tetrazolium assay (described below), similar dose response curves were obtained for BCNU, VP-16, tamoxifen, mitomycin C, methotrexate, and adriamycin against cell cultures maintained in the new medium (PDRG-basal growth medium) under ambient CO_2 or in RPMI-1640 under a 5% CO_2 environment.

In an attempt to identify a suitable, non-tetrazolium, alternative assay for use in the *in vitro* primary drug screen, a series of protein and biomass stains were investigated. The stains included seven anionic dyes that bind to the basic amino acid residues of proteins and 13 cationic dyes that bind to the negative fixed charges of biological macromolecules. Of all the reagents tested, sulforhodamine B (SRB) gave the best combination of stain intensity, signal-to-noise ratio, and linearity with cell number. SRB is a bright pink anionic dye that, in dilute acetic acid, binds electrostatically to the basic amino acids of TCA-fixed cells. Details of the SRB assay procedure are provided elsewhere.[25]

IMPLEMENTATION OF PILOT SCREENING OPERATIONS

SELECTION OF KEY ASSAY PARAMETERS: CELLULAR INOCULATION DENSITY; DRUG EXPOSURE/INCUBATION TIME

In the progression from small-scale research and development to a full-scale screen operating at a rate of > l0,000 substances tested per year, a pilot-scale screen was implemented to evaluate a number of key issues. Central among these were the selection of cell inoculation densities and assay duration, and the impact of these parameters on the choice of optimal assay methodology *(e.g.,* MTT, XTT, or SRB) for high-flux screening operations.

Under *in vitro* assay conditions, exposure to an antitumor agent may decrease the number of viable tumor cells by direct cell killing or by simply decreasing the rate of proliferation. *In vitro* assays for drug sensitivity typically employ relatively low initial cell inoculation densities *(e.g.,* a few hundred cells/well) followed by relatively long continuous drug exposure times *(e.g.,* 6–7 days, or considerably greater than the doubling times of many of the tumor panel lines). This selection of assay parameters biases toward the detection of antiproliferative effects *(i.e.,* growth inhibition), and might thereby obscure otherwise potentially interesting differential patterns of true cytotoxicity *(e.g.,* net cell killing). Moreover, with an antiproliferative or growth inhibition endpoint, cell lines with very short doubling times *(e.g.,* leukemias) will inevitably appear hypersensitive in comparison to more slowly growing tumor lines *(e.g.,* from solid tumors). There are also potential problems of nutrient deprivation, as well as practical limitations on the use of pulse drug exposures that would necessitate removal and replacement of medium. On the other hand, the longer assay duration might facilitate the detection of activity of relatively insoluble compounds or active trace constituents in mixtures or extracts. Further, the longer assay format might be essential for detection of agents that require several cell cycles for expression of lethal drug effects.

An alternative selection of assay parameters may be made in an attempt to enhance the screen's ability to discern interesting differences in true cell killing *(i.e.,* actual reduction of biomass) among the panel lines. This requires the use of a relatively large initial cell inoculum *(e.g.,* 20,000 cells/well), and a relatively short drug exposure/incubation time *(e.g.,* 1–2 days). Optimal exploitation of this format requires a high level of sensitivity and reproducibility of the assay methodology, and the capability to measure reliably the actual initial viable cell densities ("t_o" values) just prior to drug introduction.

There are reasonable arguments for or against selection of either of the two alternatives, or some compromise in between. Indeed, the NCI group has extensively investigated experimentally the impact of these assay parameters on the screen's performance, and, not surprisingly, found that certain kinds of compounds yield results that contrast greatly, depending on choice of assay parameters. However, for purposes of further studies with the pilot-scale screen, as well as for initiation of the full-scale screen, the NCI group has currently selected the high cell inoculum/short assay protocol for routine use. This selection was based principally on the desire to minimize the effects of variable doubling times of the diverse cell lines in the panel, to optimize the chances of detection of line- or

panel-specific cytotoxins, and to minimize the chances of obscuration of such effects by nonconvergent antiproliferative activities.

SELECTION OF OPTIMAL ASSAY METHODOLOGY FOR HIGH-FLUX SCREENING OPERATIONS

In order to support the choice of a tetrazolium assay (e.g., MTT or XTT) or the SRB assay for use in large-scale screening operations, a detailed, parallel screening study was performed on a common set of compounds using both MTT and SRB with the assay parameters selected above.[27] Under the experimental conditions employed and within the limits of the data analyses used, the assays gave quite comparable results. However, SRB proved to have important practical advantages for large-scale screening. Although the SRB procedure is more labor-intensive *(e.g.,* requires multiple washing steps), it has the distinct advantage of a stable end-point *(i.e.,* not time-critical, in contrast to either of the tetrazolium assays). Screening capacity, reproducibility, and quality control all appear to be markedly enhanced by adoption of the SRB for the primary screen. Therefore, the SRB assay is currently used for routine screening operations.

PILOT SCREENING EXPERIMENTS

A series of 175 known compounds, comprised of commercially marketed (NDA-approved) anticancer agents, investigational (INDA-approved) anticancer agents, and other candidate antitumor agents (compounds previously approved by the NCI Decision Network Committee for preclinical development based on activities in prior screens) were selected for pilot screening studies. The repetitive screening of these prototype "standard agents" was aimed at providing a suitable database from which a variety of novel approaches to data display and analysis could be explored. The database was also the basis for calibration and standardization of the screen, for the assessment of reproducibility of the screening data, and for the development of procedures for quality control monitoring.

In the routine Stage I screening, each agent is tested over a broad concentration range against every cell line in the current panel. All lines are inoculated onto a series of standard 96-well microtitre plates on day 0, in the majority of cases at 20,000 cells/well, then preincubated in absence of drug for 24 hours. Test drugs are then added in five tenfold dilutions starting from the highest soluble concentration, and incubated for a further 48 hours. Following this, the cells are fixed *in situ,* washed, and dried. SRB is added, followed by further washing and drying of the stained adherent cell mass. The bound stain is solubilized and measured spectrophotometrically on automatic plate readers interfaced with microcomputers, which in turn are interfaced to a mainframe computer. While the overall concept of the screen and the technical aspects of the assay methodology appear simple and straightforward, the technical, logistical, and managerial challenges to operation of the screen at the desired capacity have been great. Further details of how these challenges have been addressed are beyond the scope of this review; however, they will be provided in a separate paper.[26] Currently, the primary screening laboratory is capable of testing compounds at a rate of 400 per week against a 60-line panel.

APPROACHES TO DATA DISPLAY AND ANALYSIS

GENERAL CONSIDERATIONS

The testing of each compound in the primary screen generates a voluminous amount of data, the analysis of which also presents unique challenges. In its simplest graphical presentation, each test generates a series of dose-response curves *(e.g.,* one curve, five concentration points for each cell line). The shapes of the dose response curves may be relatively simple *(e.g.,* monophasic) or complex *(e.g.,* multiphasic) and may vary from line to line and/or compound to compound. Moreover, the effects of t_z calculations may be either relatively inconsequential or profound depending on the compound, the line(s), and/or the kind of numerical or graphical analyses employed. The development of optimal strategies for analysis and display of the screening data, and the identification of optimal criteria for selection and prioritization of novel active compounds, are currently subjects of intensive investigation and are beyond the scope of this overview. However, even some relatively simplistic approaches, one example of which is described briefly below, appear to offer an intriguing preview into the potential of the new screen to identify reliably known active antitumor agents, as well as new antitumor leads.

THE MEAN GRAPH

One simple way to obtain a measure of the relative cell line sensitivities to a given drug is to compare the relative drug concentrations required to produce the same level of response *(e.g.,* IC_{10}s; IC_{50}s; lC_{90}s) in each cell line. In order to create a visual means to facilitate this kind of comparison, the "mean graph" display was developed.[30] While the mean graph data display format can be applied to many different indices of cellular responsiveness, examples considered here are limited to those based on IC_{50} values. A typical computer-constructed mean graph, derived from IC_{50} values, is centered at the arithmetic mean of the logarithm of the IC_{50} values for all cell line responses measured for the given compound. The choice of the mean as an anchor point is arbitrary; nevertheless, it may also be similarly useful for development of other mean-graph-derived analyses, such as the estimation of relative cell line sensitivities.[31]

FIG. 3. IC_{50} Mean Graphs from screening of 5-fluorouracil (*A*), 5-fluorodeoxyuridine (*B*), and bleomycin (*C*). Individual $\log_{10}$ IC_{50s} for each cell line are shown only for (*A*). Mean log IC_{50s} for (*A*), (*B*) and (*C*), respectively, were −3.5 (*A*); −4.7 (*B*); −5.2 (*C*).

A typical mean graph is shown in Figure 3A. It is constructed by projecting bars, one for each cell line (listed by identifier on the left of the graph), to the right or left of the mean, depending on whether cell sensitivity to the test drug is greater or less than average, respectively. The length of a bar is proportional to the difference between the logarithm of the cell line IC_{50} and the mean. For example, for a given cell line, a bar projecting 3 log units to the right of the mean *(e.g.,* as for P-388 in Figure 3A) would reflect an individual cellular sensitivity 1,000 times that of the average of all of the cellular responses to the given compound represented on the graph. Thus, if the compound

depicted in Figure 3A had been screened as an unknown, the very sensitive P-388 line, and perhaps also H-460, might reasonably have been selected for follow-up *in vivo* testing. Moreover, since the colon cancer lines as a group appeared relatively sensitive, some *in vivo* xenograph testing with one or more of these lines might logically have been undertaken. Using the information from the primary screen in this manner, it is probable that we would have "discovered" 5-fluorouracil, the compound that yielded the mean graph shown in Figure 3A.

Figure 3B shows another interesting mean graph. In this instance, P-388 and H-460 again appeared quite sensitive to the test drug. Indeed, based on our current experience from the testing of several thousand diverse compounds, P-388 continues to show the highest overall average sensitivity compared to all other lines in the present 60-line panel.[31] Not far behind in overall average sensitivity are the H-460 lung line, the LOX melanoma, and most of the human leukemia lines.

The interesting feature of the differential cellular response fingerprint in Figure 3B is the unusually high sensitivity of several of the brain tumor lines that normally are quite highly resistant to most agents tested in the *in vitro* panel. The drug that yielded this mean graph is 5-flurodeoxyuridine (FUDR). Is this a "new look" at an "old drug" that should be explored further? Has FUDR ever been adequately tested in the clinic against brain cancers? This same kind of question may apply to other agents that may be contained in our current study set of NDA and INDA drugs, as well as other agents for which there already may be some clinical experience, irrespective of whether or not such agents have previously been deemed "clinically active" or "inactive." In this respect, the more detailed analysis of the new screening data, as well as the available clinical information, on "old" agents will be an area of immediate priority.

Given the typically high apparent sensitivities of the P-388 and the human leukemia cell line panel in the mean graph analyses of the majority of known antitumor agents, the mean graph shown in Figure 3C offers a striking contrast. With this drug, most of the leukemia lines are much less sensitive than the normally resistant solid tumor lines. This very rare type of fingerprint certainly would have led to the selection of this agent for follow-up *in vivo* testing against a selection of the sensitive solid tumor lines in the panel. It is interesting that the drug that produced Figure 3C is bleomycin, an agent that is not active *in vivo* against P-388 (nor L1210) in the mouse, nor is it very effective clinically against human leukemias. It is also interesting that bleomycin is not myelotoxic or immunosuppressive, whereas most drugs active clinically against leukemias and/or preclinically against P-388 are marrow poisons.

The above examples are provided for illustrative purposes and by no means represent a comprehensive or conclusive analysis of performance and utility of the new *in vitro* screen. The mean graph is but one simplistic approach to exploiting the wealth of information potentially available from the screen. While intriguing, it must still be cautioned that different kinds of approaches to data analysis and interpretation could lead to rather different conclusions, strategies, and priorities for follow-up investigations. Nevertheless, we can conclude from our current experience that, simplistic as they are,

the mean graph fingerprints for almost all of the known active antitumor agents thus far evaluated are highly reproducible and highly characteristic for individual agents, and, in some cases, for subsets of chemically and/or biologically related agents. These observations have been facilitated by the development and use of a computerized pattern-recognition algorithm as described and illustrated below.

COMPARE

A computer program called COMPARE was developed to explore further differences and similarities among the mean graph fingerprints of compounds contained in the standard agent database.[30] COMPARE can be used to rank the similarity of the mean graph pattern of a specified "seed" compound to the patterns of all the other compounds in the NCI screening database or some defined subset thereof. Any previously tested compound can be used as the seed to initiate the pattern-recognition algorithm.

Application of COMPARE to some prototypical seed compounds yielded some very intriguing observations. For example, when doxorubicin was used as the seed, COMPARE consistently ranked as highly comparable the fingerprints of a number of other DNA-binders and topoisomerase inhibitors. This is exemplified in Figure 4A–C, which shows the remarkable similarities of the mean graphs produced by adriamycin, rubidazone, and daunomycin.

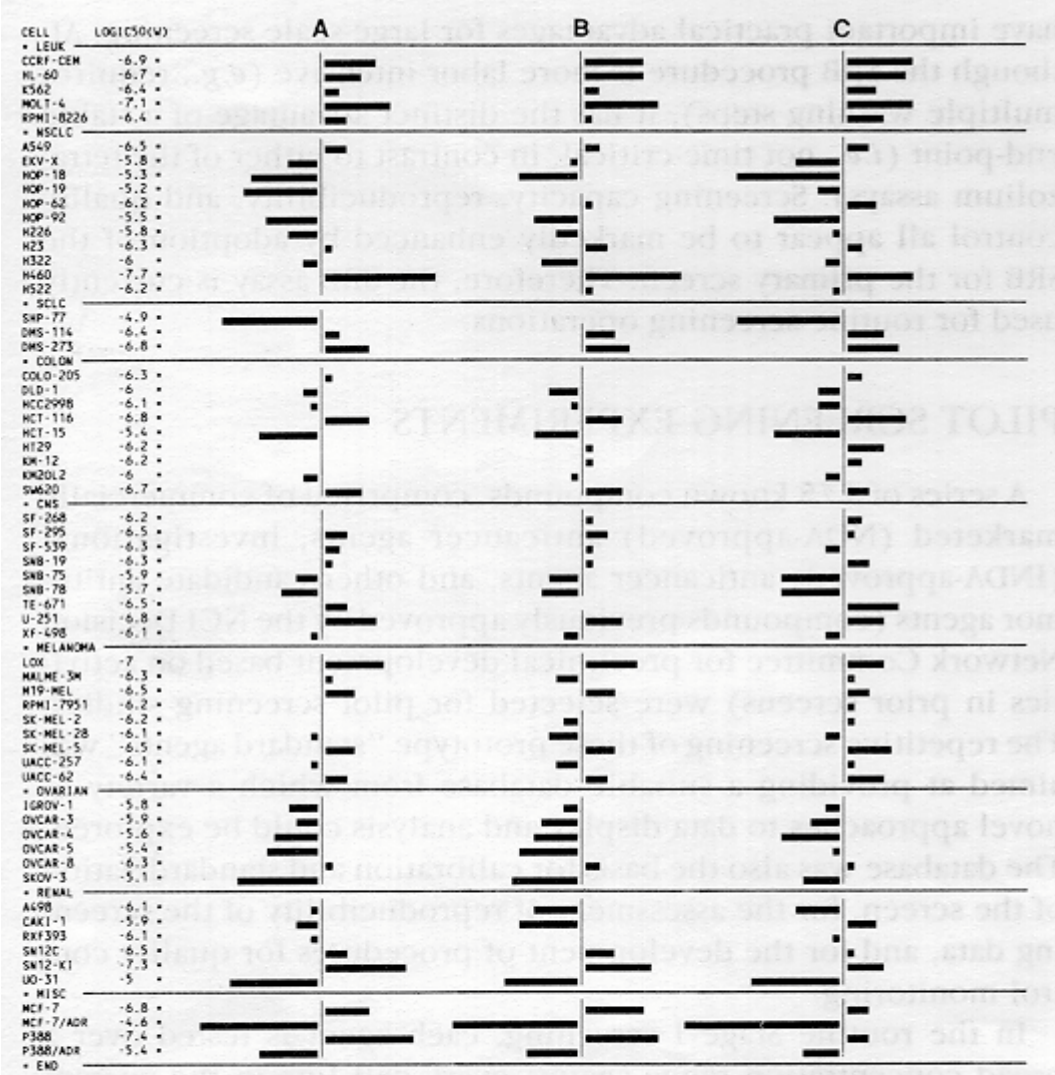

FIG. 4. IC_{50} Mean Graphs from screening of doxorubicin (*A*), rubidazone (*B*), and daunomycin (*C*). Individual $\log_{10}$ IC_{50s} for each cell line are shown only for (*A*). Mean log IC_{50s} for (*A*), (*B*), and (*C*), respectively, were −6.2 (*A*); −5.8 (*B*); −6.5 (*C*).

When known alkylating agents were used as the seeds, the COMPARE analysis invariably showed other alkylating agents in the database as most highly comparable. For example, Figure 5A-C shows the striking similarities of mean graphs produced by chlorambucil, thiotepa, and triethylenenmelamine, which were ranked most similar to each other irrespective of which of the three was used as the "seed." The COMPARE analysis further showed that antimetabolites and several other mechanistically related subsets of agents also tended to group to a significant extent with agents having similar activity or structure.

Do all compounds tested give distinctive mean graph patterns of differential cellular sensitivity? The answer is most assuredly negative. Indeed, most randomly selected substances give essentially flat, featureless mean graph profiles, due either to the fact that they are devoid of any apparent effects on the cells or that they indiscriminantly kill or inhibit the growth of all cells in the panel in a non-differential manner. Moreover, not surprisingly, certain types of clinically useful antitumor drugs are quite inactive in

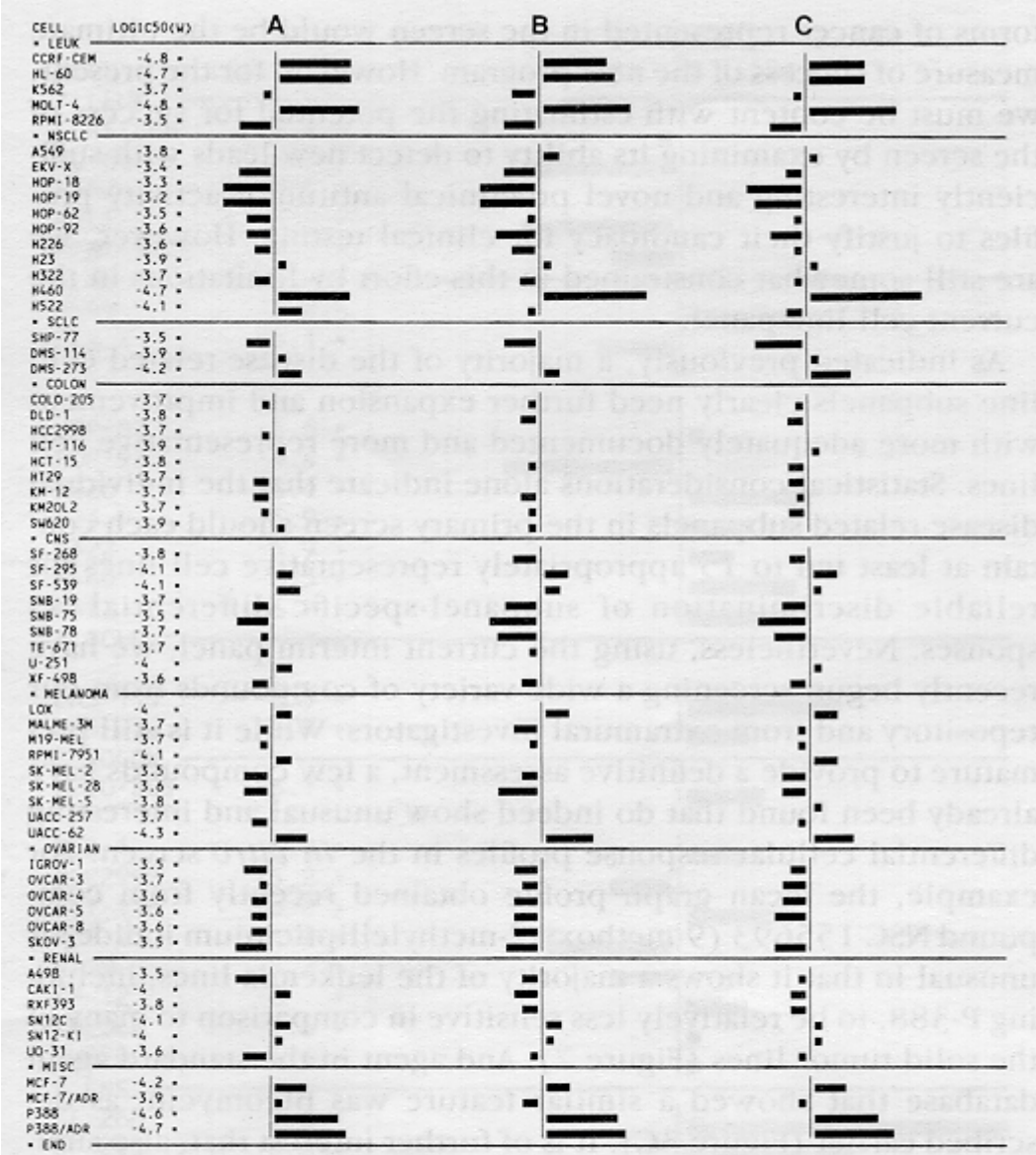

FIG. 5. IC_{50} Mean Graphs from screening of chlorambucil (*A*), thiotepa (*B*), and triethylenemelamine (*C*). Individual $\log_{10} IC_{50s}$ for each cell line are shown only for (*A*). Mean log IC_{50s} for (*A*), (*B*), and (*C*), respectively, were −3.8 (*A*); −3.7 (*B*); −4.7 (*C*).

the screen. Examples of some of these are shown in Figure 6A-C and include cyclophosphamide, procarbazine, and hexamethylmelamine; such compounds, which require *in vivo* metabolic activation, will not be detected by the *in vitro* screen.

The apparent robustness of mean graph fingerprints to reflect consistently certain differences or similarities in biological properties and/or chemical structure appears to offer potential utility in the standardization and quality control monitoring of the screen. The day-to-day performance of individual cell lines in the screen can be monitored with

respect to some selected level of response *(e.g.,* IC_{50}) to a standard agent of choice. However, for purposes of this particular screen, the performance of individual cell lines is not so important as is how they all respond relative to each other. In this respect, it appears that we can exploit further the COMPARE analysis. For example, as a matter of routine, we currently screen once every month a fixed subset of approximately 40 standard agents ("monthly standards"). From these data we can use the COMPARE analysis to monitor consistency of the mean graph fingerprints over time. Moreover, one or two compounds selected from the monthly standards are screened daily ("daily standards"). COMPARE analysis of the results from the daily standards against the monthly standard database can be used as a means to determine that the screen is performing adequately *(i.e.,* giving the correct "fingerprints" of the standards) on any given day. If the fingerprint of a daily standard does not match closely with the correct fingerprints in the monthly standard database, the respective day's entire screening data are viewed as suspect or invalid until proven otherwise.

STRATEGIES FOR SELECTION OF NEW ACTIVE LEADS

NEW CLINICAL CANDIDATES VS. NEW ACTIVE LEADS

As already described above, it is conceivable that the new screen may help identify "old drugs" *(i.e.,* compounds that have already reached the stage of testing or therapeutic use in the clinic) that merit testing in specific forms of cancer for which previous testing is inconclusive. However, the list of "old drugs" is not large. Indeed, the main purpose for the new screen is to identify entirely new candidate antitumor agents ("new leads") for more detailed preclinical investigation that might lead to their further development and possible candidacy for clinical trial. Only after a new lead selected by the screen has subsequently negotiated successfully the demanding pathways of preclinical development can it be considered a new clinical candidate. Nevertheless, it is appropriate to consider what kinds of new clinical candidates we might anticipate seeing in the future as the result of the implementation of the new screen.

DISCOVERY OF CHEMICAL OR BIOLOGICAL ANALOGS OF KNOWN ANTITUMOR AGENTS

It seems already apparent that the new screen can be utilized in a compound-oriented strategy at least as effectively as with prior screens, to discover new agents with biological activity profiles analogous to currently known antitumor drugs. For example, a medicinal chemist might wish to prepare new derivatives of a known prototype drug that might have improved physicochemical properties, or lower toxicity or otherwise potentially improved features, yet that retained the desired highly characteristic differential cellular response "fingerprint" produced by the prototype. The desired prototypes might be selected from among the known clinically active drugs whose screening profiles are contained in the standard agent database. Alternatively, the chemist may wish to rationally conceive and synthesize an entirely new class of compounds *(e.g.,* which he may attempt to design to act by the same mechanism as the chemically unrelated prototype) that would produce the same biological fingerprint *(i.e.,* a "biological analog") as the desired prototype. Or, lacking any preconceived notion of what kinds of new structural classes might act as biological analogs, he may simply wish

to empirically screen widely diverse compounds to discover new leads giving the same cellular response profile as the selected prototype. Similarly, a natural product chemist might seek to isolate and identify new active agents from a complex extract that showed an *in vitro* screening activity profile suggestive of the presence of a known active prototype or some unknown chemical or biological analog thereof. Or, he might wish to eliminate from further pursuit those extracts giving an all-too-familiar fingerprint; that is to say, the screen may offer a new avenue for dereplication.

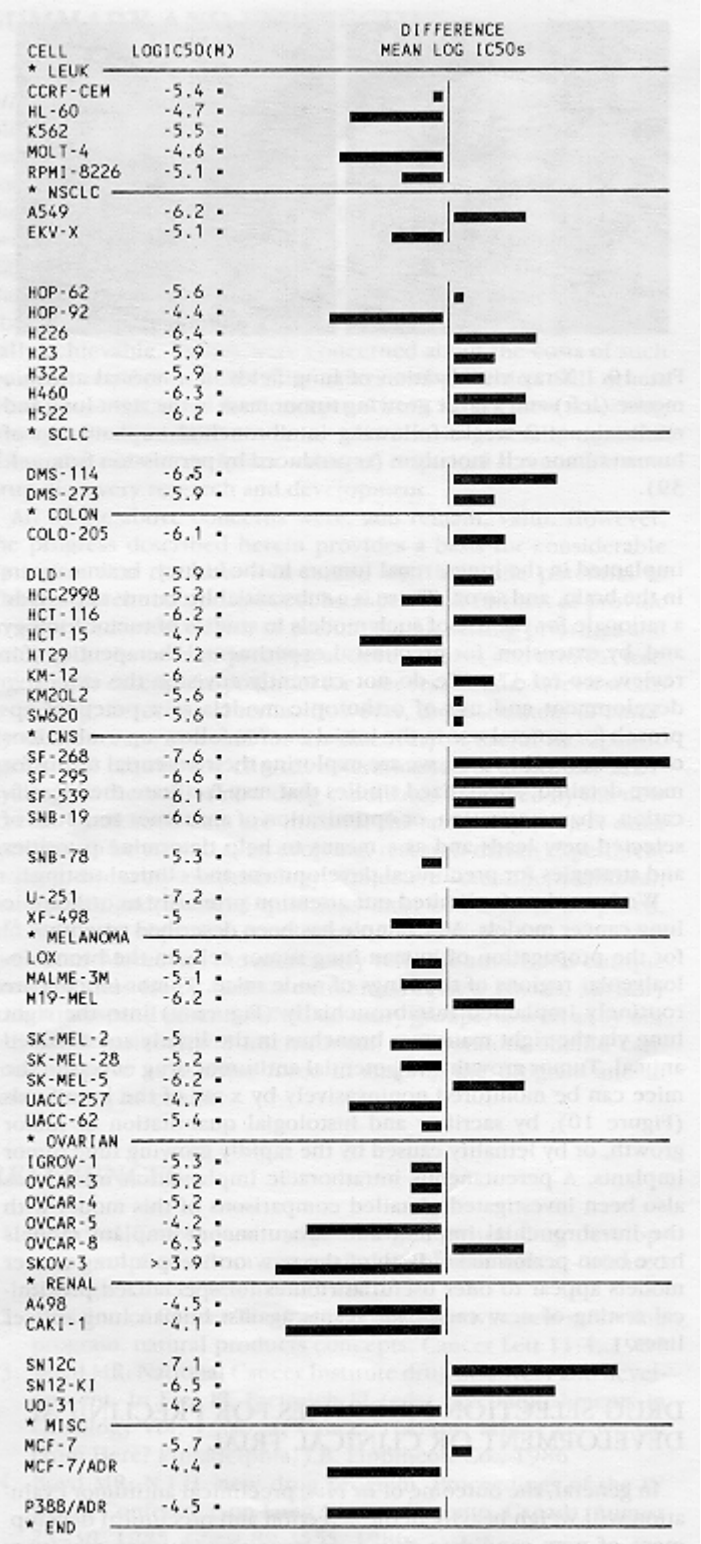

FIG. 7. Mean Graph obtained from screening of compound NSC 155693. The mean $\log_{10}$ IC_{50} for this compound was −5.5. Individual IC_{50} values for each cell line are shown.

Tools and techniques such as the mean graph and the COMPARE program might be applicable to the above. For example, the mean graph of the desired prototype compound might he used as the "seed" and the desired databases obtained from the screening of new compounds could be searched for new leads giving the desired biological fingerprint.

Thus, there seems little doubt that we can exploit the new screen effectively as above to discover "more or the same," with respect to the currently limited spectrum of available clinically useful prototype drugs. However, the main incentive for development of the new screen was to provide an important new dimension for the discovery of entirely new kinds of active antitumor agents quite unlike any of the current prototypes and that would have gone undetected by prior screens.

DISCOVERY OF NEW BIOLOGICAL CLASSES OF ANTITUMOR AGENTS

The above compound-oriented (chemistry-driven) strategy emphasizes a search for new compounds that produce preselected biological response profiles. The main goal of the new screen is to provide us an unprecedented opportunity to pursue a biology-driven *(e.g.,* disease-oriented) drug screening strategy. With this strategy, the emphasis is upon the search for new biological activity profiles of interest; it is less relevant whether the compound producing such a profile is of a known or a "new" chemical structure. It is important to determine as soon as possible whether the new screen can serve this goal.

Certainly the discovery of a new agent that proved to have important new clinical utility against any or all of the various forms of cancer represented in the screen would be the ultimate measure of success of the new program. However, for the present, we must be content with estimating the potential for success of the screen by examining its ability to detect new leads with sufficiently interesting and novel preclinical antitumor activity profiles to justify their candidacy for clinical testing. However, we are still somewhat constrained in this effort by limitations in the current cell line panel.

As indicated previously, a majority of the disease-related cell-line subpanels clearly need further expansion and improvement with more adequately documented and more representative cell lines. Statistical considerations alone indicate that the individual disease-related subpanels in the primary screen should each contain at least ten to 15 appropriately representative cell lines for reliable discrimination of subpanel-specific differential responses. Nevertheless, using the current interim panel, we have recently begun screening a wide variety of compounds from our repository and from extramural investigators. While it is still premature to provide a definitive assessment, a few compounds have already been found that do indeed show unusual and interesting differential cellular response profiles in the *in vitro* screen. For example, the mean graph profile obtained recently from compound NSC 155693 (9-methoxy-2-methylellipticinium iodide) is unusual in that it shows a majority of the leukemia lines, including P-388, to be relatively less sensitive in comparison to many of the solid tumor lines (Figure 7). And agent in the standard agent database that showed a similar feature was bleomycin, as described earlier (Figure 3C). It is of further interest that, also similar to bleomycin, the compound NSC 155693 showed minimal *in vivo* activity against P-388 (or L1210). However, in contrast to bleomycin, NSC 155693 shows an apparent clustering of *in vitro* activity among the normally very resistant brain cancer lines and non-small cell lung cancer lines. We are currently subjecting this compound and a number of other new leads to detailed *in vivo* preclinical testing. The strategy for such testing is outlined briefly below.

IN VIVO PRECLINICAL TESTING OF NEW LEADS

GENERAL CONSIDERATIONS

The data available from the *in vitro* primary screen uniquely provide us the basis for selection of the most appropriate tumor lines to use for follow-up *in vivo* testing; it is unnecessary to test *in vivo* an interesting compound against all of the panel lines, only those most sensitive or otherwise of particular interest. All of our *in vivo* testing with human tumor lines is performed in athymic nude mice. The emphasis is on the treatment of animals bearing fully established tumors, where robust endpoints of tumor lysis or regression can be sought. This is in contrast to the antiproliferative or growth-inhibitory endpoints that are typically measured when *in vivo* tumor inocula and drug administrations are performed simultaneously and/or into the same body compartment. *In vivo* drug testing protocols are individually tailored to each individual compound/cell line/test model combination. Special *in vitro* studies, as well as parallel pharmacological and toxicological investigations, are used to optimize the choices of schedule and dose. In short, each new lead for *in vivo* evaluation is not subjected to a rigidly predefined set of protocols, but instead is approached with an individualized research orientation. This is particularly important because of the widely diverse biology and growth characteristics of the tumor lines of potential interest, as well as the specialized models that might be employed. Model systems that are being explored for *in vivo* investigations with panel lines include a microencapsulated tumor model, subcutaneous xenograft models, and orthotopic models.

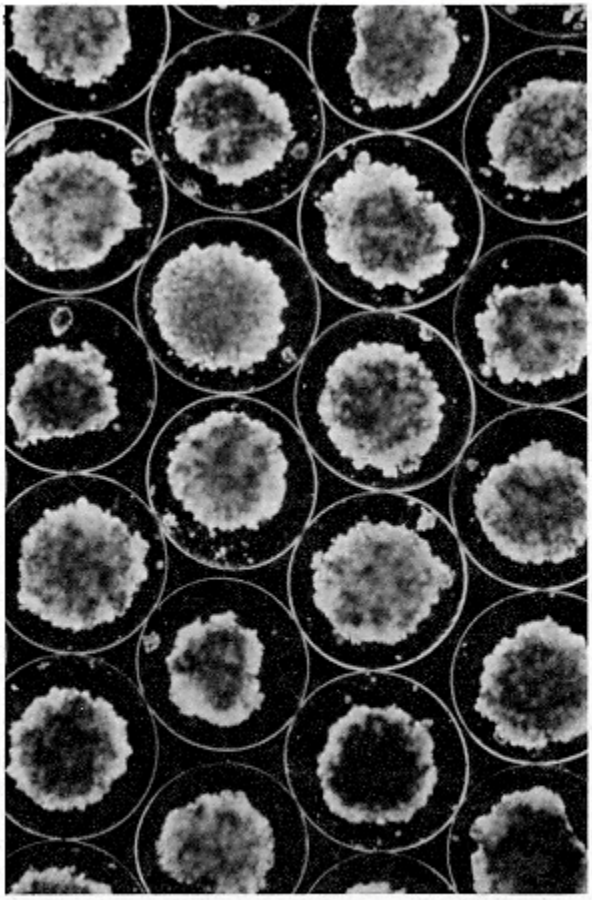

FIG. 8. Microencapsulated human tumor cells for *in vivo* implantation. Individual microcapsules average approximately 1 mm in diameter. (Photomicrograph provided courtesy of Dr. James McMahon, NCI)

THE MICROENCAPSULATED TUMOR ASSAY

As one approach to development of an *in vivo* model that might be useful with a wide diversity of tumor types, a microencapsulated tumor assay (META) has been investigated. The assay employs a proprietary microencapsulation technology,[32] and makes it possible to encapsulate human tumor cells in small microcapsules *(e.g.,* see Figure 8) that can be inoculated directly into the peritoneal cavity of nude mice. The

semipermeable membranes of the microcapsules are intended to confine and protect the tumor cells, but permit the flow of nutrients required for *in vivo* growth. Test drugs can be administered systemically, microcapsules recovered at the desired times, and drug effects assessed by comparisons of viable tumor cell mass in microcapsules recovered from treated vs. control animals.

The general concept for this assay is interesting and potentially attractive; however, our experiences to date suggest that, in the desired *in vivo* drug screen application, problems with reproducibility and other technical and practical constraints preclude its routine use in the NCI program. Any application of the META will likely be limited to specialized experiments carried out under rigidly controlled and defined research laboratory conditions not readily achievable in a busy *in vivo* testing environment.

THE SUBCUTANEOUS XENOGRAFT MODEL

Many of the current cell panel lines have already been adapted and characterized for growth as xenografts in nude mice for *in vivo* drug evaluation. Moreover, this continues to be an area of further intensive development, as the cell line panel continues to be expanded and refined. Indeed, the subcutaneous xenograft model will likely provide the mainstay for the *in vivo* testing counterpart to the NCI *in vitro* drug discovery screen. This model is readily amenable to the determination of *in vivo* drug effects directly on fully established mass tumors derivable from many different human tumor lines of interest. Thus far, colon and melanoma lines have proved to be most readily established *in vivo,* whereas brain tumors have been most difficult. Nevertheless, even among the brain tumor lines, successful subcutaneous xenografts have been developed for about half of the lines currently available. Xenografts have also been established for a majority of the current renal cancer lines, melanoma lines, ovarian cancer lines, and lung cancer lines. Volume doubling times have been determined for all of the xenografts to date, and generally range from about 2 days to 2 weeks or greater.

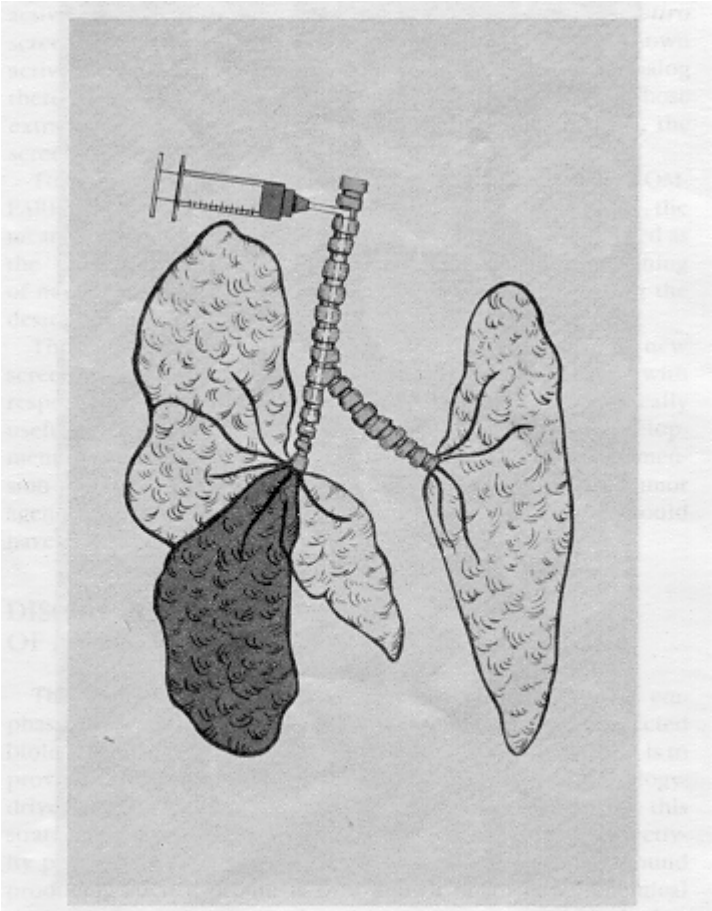

FIG. 9. Intrabronchial tumor cell implantation technique for orthotopic *in vivo* human lung cancer model in athymic nude mice (reproduced by permission from ref. 39).

THE ORTHOTOPIC XENOGRAFT MODEL

With "orthotopic" models, the

human tumor cells are implanted at the organ sites analogous to the presumed site of origin of the respective tumor implant. For example, lung tumors are implanted in the lungs, renal tumors in the kidney, brain cancers in the brain, and so on. There is a substantial literature to provide a rationale for the use of such models in studies of tumor biology and, by extension, for preclinical experimental therapeutics (for review see ref.37). We do not currently envision the extensive development and use of orthotopic models as a practical approach for general use in the initial *in vivo* follow-up evaluations of new leads. However, we are exploring their potential utility for more detailed, specialized studies that may facilitate the identification, characterization, or optimization of antitumor activities of selected new leads and as a means to help determine priorities and strategies for preclinical development and clinical testing.

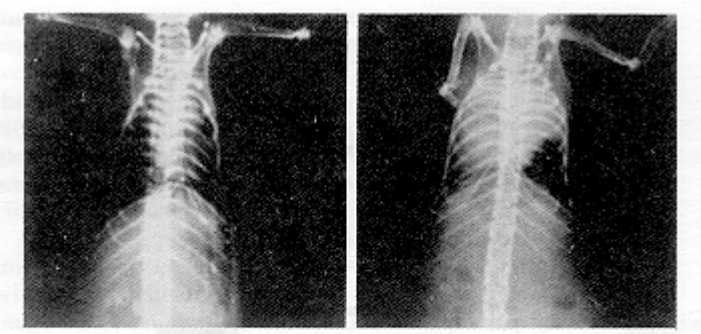

FIG. 10. X-ray visualization of lung fields of a normal athymic mouse (left) and a large growing tumor mass in the right lung and mediastinum 2 weeks following intrabronchial implantation of human tumor cell inoculum (reproduced by permission from ref. 39).

We have thus far limited our attention primarily to orthotopic lung cancer models. An example has been described recently[38,39] for the propagation of human lung tumor cells in the bronchioloalveolar regions of the lungs of nude mice. Tumor inocula are routinely implanted intrabronchially (Figure 9) into the right lung via the right mainstem bronchus in the lightly anesthetized animal. Tumor growth and potential antitumor drug effects in the mice can be monitored noninvasively by x-ray of the lung fields (Figure 10), by sacrifice and histologial quantitation of tumor growth, or by lethality caused by the rapidly growing hung tumor implants. A percutaneous intrathoracic implantation model has also been investigated; detailed comparisons of this model with the intrabronchial implant and subcutaneous implant models have been performed.[39] Both of the new orthotopic lung cancer models appear to offer useful attributes for specialized preclinical testing of new candidate agents against human lung cancer lines.

DRUG SELECTION PRIORITIES FOR PRECLINICAL DEVELOPMENT OR CLINICAL TRIAL

In general, the outcome of *in vivo* preclinical antitumor evaluation will weigh heavily in the selection and preclinical development of new candidate drugs. As with the *in vitro* discovery screen, novelty or uniqueness of the *in vivo* antitumor activity profile will be an important consideration. Finally, for compounds completing preclinical development, recommendations for clinical trials will likewise give priority to interesting new agents with antitumor activities most distinctly contrasted from those of agents selected by prior screens. In this way, we hope most rapidly and efficiently to validate, or disprove, the

ability of the new screen to discover effective new drugs to complement those currently available.

SUMMARY AND PERSPECTIVE

The technical and operational feasibility of the new NCI *in vitro* primary antitumor drug screen is now established. The implementation of the complementary *in vivo* counterpart for follow-up evaluation of new leads is well underway. While simple in concept, the scientific, technical, and managerial challenges to the development and implementation of the new screen have proved even greater than any of us involved in the program would likely have originally envisioned. Moreover, from the beginning, the project has been a subject of controversy. Some concerned observers simply doubted that the screen as proposed was technically achievable. Others were concerned about the costs of such an effort relative to other alternatives and priorities. Still others were unconvinced of the conceptual basis for the new screening strategy. Finally, there are those who remain generally doubtful about the current or future value of empirical screens for new drug discovery research and development.

All of the above concerns were, and remain, valid. However, the progress described herein provides a basis for considerable optimism that the new NCI screen will have the potential to identify novel new antitumor substances, and, further, to provide an unprecedented wealth of useful data for setting priorities and guiding the courses of preclinical development and clinical testing. To this extent, the potential for "success" of the screen seems promising, in technical terms. However, the assessment of *validity* of the screen as a tool for the discovery of useful therapeutic agents will require the clinical determination of antitumor activity, or lack thereof, of new drug candidates identified by the new screen. Until such data are in hand, the screening project must continue to be viewed as an evolving, research-driven experiment in drug discovery methodology. Objective scientific, technical, managerial, and financial questions must continue to be posed, and similarly objective conclusions and decisions to be drawn therefrom. Moreover, the immensely valuable advice and critique provided thus far by various extramural review boards, advisory committees, and other non-NCI advisory groups, as well as by our individual national and international collaborators, should continue to serve as a major force in shaping the program and in determining its course.

REFERENCES

1. National Cancer Institute planning to switch drug development emphasis from compound to human cancer-oriented strategy. Cancer Lett 10:1, 1984
2. Division of Cancer Treatment Board approves new screening program, natural products concepts. Cancer Lett 11:4, 1985

3. Boyd MR: National Cancer Institute drug discovery and development. In Frei EJ, Freireich EJ (eds): Accomplishments in Oncology Vol. 1, No. 1, Cancer Therapy: Where Do We Go From Here? Philadelphia, J.B. Lippincott Co., 1986
4. Boyd MR: N.I.H. new drug program. Proceedings of the IV World Conference on Lung Cancer, Toronto, Canada, August 25–30, 1985. Chest 89:355S, 1986
5. Workshop on "Disease-oriented Antitumor Drug Discovery and Development," NIH, Bethesda, MD, January 9–10, 1985. Sponsored by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute
6. *Ad Hoc* review committee proceedings for National Cancer Institute *In Vitro/In Vivo* Disease-oriented Screening Project. NIH, Bethesda, MD, September 23–24, 1985. Sponsored by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute
7. *Ad Hoc* review committee proceedings for National Cancer Institute *In Vitro/In Vivo* Disease-oriented Screening Project. NIH, Bethesda, MD, December 8–9, 1986. Sponsored by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute
8. *Ad Hoc* review committee proceedings for National Cancer Institute *In Vitro/In Vivo* Disease-oriented Screening Project. NIH, Bethesda, MD, December 8–9, 1989. Sponsored by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute
9. Boyd MR, Shoemaker RH, Cragg GM, Suffness M: New avenues of investigation of marine biologicals in the anticancer drug discovery program of the National Cancer Institute. In: Jefford CW, Rinehart KL, Shield US (eds): Pharmaceuticals and the Sea. Lancaster, Technomic Publishing AG, 1988
10. Boyd MR, Shoemaker RH, McLemore TL, et al: New drug development. In: Roth JA, Ruckdeschel JC, Weisenburger TH (eds): Thoracic Oncology. Philadelphia, W.B. Saunders Co, 1989
11. Boyd MR: Status of implementation of the NCI human tumor cell line *in vitro* primary drug screen. Proc Am Assoc Cancer Res30:652, 1989
12. Driscoll JS: The preclinical new drug research program of the National Cancer Institute. Cancer Treat Rep 68:63, 1984
13. Shoemaker RH, Wolpert-DeFilippes MK, Kern DH, et al: Application of a human tumor colony-forming assay to new drug screening. Cancer Res 45:2145, 1985
14. Shoemaker RH, Monks A, Alley MC, et al: Development of human tumor cell line panels for use in disease-oriented drug screening. In Hall T (ed): Prediction of Response to Cancer Chemotherapy. New York, Alan Liss, 1988
15. Donovick R, Alley M, Stinson S, et al: Current status and future development of disease-oriented panels of human tumor cell lines for use in the NCI anticancer drug screen. Proc Am Assoc Cancer Res 30:611, 1989
16. Stinson SF, Alley MC, Kenney S, et al: Morphologic characterization of Human Carcinoma Cell Lines. Proc Am Assoc Cancer Res 30:613, 1989
17. Alley MC, Hursey ML, Pacula-Cox CM, et al: Suitability of multicellular growth units in soft agar culture for experimental drug evaluations and morphologic examinations. Proc Am Assoc Cancer Res 30:529, 1989

18. McLemore T, Alley M, Liu M, et al: Histopathologic, biochemical and molecular genetic characterization of four newly established human pulmonary carcinoma cell lines. Proc Am Assoc Cancer Res 30:225, 1989
19. McLemore T, Storeng R, Adelberg S, et al: Expression of different cytochrome P450 genes in human hung cancer cell lines. Proc Am Assoc Cancer Res 30:11, 1989
20. Alley MC, Scudiero DA, Monks A, et al: Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 48:589, 1988
21. Paull KD, Shoemaker RH, Boyd MR, et al: The synthesis of XTT: A new tetrazolium reagent that is bioreducible to a water-soluble formazan. J Heterocyclic Chem 25:911, 1988
22. Scudiero DA, Shoemaker RH, Paull KD, et al: Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res 48:4827, 1988
23. Vistica DT, Skehan P, Scudiero DA, et al: Tetrazolium-based assays for cellular viability: A critical examination of parameters which affect formazan production. Proc Am Assoc Cancer Res30:612, 1989
24. Vistica DT, Scudiero DA, Skehan P, et al: Development and evaluation of a CO_2-independent culture medium for use in a high-flux *in vitro* anticancer drug screen employing a broad panel of human tumor cell lines. Proc Am Assoc Cancer Res 30:613, 1989
25. Skehan P, Storeng R, Scudiero N, et al: Evaluation of colon-metric protein and biomass stains for assaying *in vitro* drug effects upon human tumor cell lines. Proc Am Assoc Cancer Res 30:612, 1989
26. Monks A, Scudiero D, Skehan P, Boyd M: Implementation of a pilot-scale, high flux anticancer drug screen utilizing disease-oriented panels of human tumor cell lines in culture. Proc Am Assoc Cancer Res 30:607, 1989
27. Rubinstein LV, Paull KD, Shoemaker RH, et al: Correlation of screening data generated with a tetrazolium assay (MIT) versus a protein assay (SRB) against a broad panel of human tumor cell lines. Proc Am Assoc Cancer Res 30:607, 1989
28. Boyd MR: Strategies for the identification of new agents for the treatment of AIDS: A national program to facilitate the discovery and preclinical development of new drug candidates for clinical evaluation. In: DeVita VT Jr, Hellman S, Rosenberg SA (eds): AIDS: Etiology, Diagnosis, Treatment and Prevention. Philadelphia; J. B. Lippincott Company, 1988
29. Weislow OS, Kiser R, Fine DL, et al: New soluble-formazan assay for HIV-1 cytopathic effects: Application to high-flux screening of synthetic and natural products for AIDS-antiviral activity. J Natl Cancer Inst 81:577, 1989
30. Paull KD, Shoemaker RH, Hodes L, et al: Display and analysis of patterns of differential activity of drugs against human tumor cell lines: Development mean graph and COMPARE algorithm. J Nath Cancer Inst 81:1088, 1989
31. Paull KD, Hodes L, Plowman J, et al: Reproducibility and response patterns of the IC_{50} values and relative cell line sensitivities from the NCI human tumor cell line drug screening project. Proc Am Assoc Cancer Res 29:488, 1988

32. Gorelik E, Ovejera A, Shoemaker R, et al: Microencapsulated tumor assay: New short-term assay for *in vivo* evaluation of the effects of anticancer drugs on human tumor cell lines. Cancer Res 47:5739, 1987
33. McMahon J, Camalier R, Shoemaker R, et al: Evaluation of feasibility of microencapsulation technology for the evaluation of anti-HIV drugs *in vivo.* The FASEB Journal 2:A913, 1988
34. Shoemaker RH, McLemore TL, Abbott BJ, et al: Human tumor xenograft models for use with an *in vitro-based,* disease-oriented antitumor drug screening program. In: Pinedo HM, Peckham MJ, Winograd B (eds): European School of Oncology Monograph: Human Tumor Xenografts in Anticancer Drug Development. Berlin, Springer-Verlag, 1988
35. Fiebig HH, Winterhalter B, Berger D, et al Properties of 6 human tumor xenografts *in vivo* from which cell lines were developed. Proc Am Assoc Cancer Res 30:612, 1989
36. Dykes DJ, Mayo JG, Abbott BJ, et al: *In vivo* growth characteristics of human tumor xenografts from the NCI *in vitro* "disease-oriented" drug discovery program. Proc Am Assoc Cancer Res 30:6 14, 1989
37. Fidler IJ: Rationale and methods for the use of nude mice to study and biology and therapy of human cancer metastasis. Cancer Metastatis Rev 5:29, 1986
38. McLemore TL, Abbott BJ, Mayo JG, Boyd MR: Development and application of new orthotopic *in vivo* models for use in the U.S. National Cancer Institute's drug screening program. In: Wu B, Zheng JS (eds): 6th International Workshop on Immunodeficient Animals in Biomedical Research. Basel: S Karger AG, 1988
39. McLemore TL, Liu MC, Blacker PC, et al: Comparison of intrapulmonary, percutaneous intrathoracic, and subcutaneous models for the propagation of human pulmonary and nonpulmonary cancer cell lines in athymic nude mice. Cancer Res 48:1880, 1988